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THE PHYTOPLANKTON OF THE GULF OF CALIFORNIA OBTAINED BY THE "E. W. SCRIPPS" IN 1939 AND 1940*

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The waters of the Gulf of California are fabulously rich in marine life; they fairly teem with multitudes of fish of both commercial and sport varieties. To maintain these large numbers there must be correspondingly huge crops of their ultimate food—the phytoplankton. And, indeed, it was the enormous quantity of these microscopic plants that gave the Gulf its early and descriptive name—the Vermilion Sea. In 1939 and again in 1940 the "E. W. Scripps," research vessel of the Scripps Institution of Oceanography of the University of California, spent several months in these waters and among the data collected on these cruises were quantitative samples of this phytoplankton. It is with these samples that the present report deals.

The phytoplankton of the Gulf, or rather the diatoms which in this region are its main constituent, also plays a considerable geologic role. Bottom samples taken on these two cruises revealed that diatomaceous sediments are being laid down over large areas in the Gulf, probably under conditions similar to those which gave rise to the Miocene diatomaceous shales of the California coast region (Sverdrup and Staff, 1940).

* Contribution from the Scripps Institution of Oceanography, New Series, No. 183.

Prior to 1939 four series of phytoplankton samples had been collected in the Gulf of California. The first series was obtained in 1921 between April 7 and July 11 by the Expedition of the California Academy of Sciences (Allen, 1923); the second in November of 1935 by the Templeton Crocker Expedition (Allen, 1938); the third in 1936 between February 8 and March 20 by the Allan Hancock Expedition of 1936 (Allen, 1937); and the fourth by the Allan Hancock Expedition of 1937 (Cupp and Allen, 1938) between March 1 and April 4.

The 1939 cruise (Figure 30) of the "E. W. Scripps" was of a general oceanographical nature and between February 13 and March 19 the ship occupied 53 stations, which were spaced 15 to 20 miles apart along 9 lines across the Gulf; at 49 of these stations phytoplankton samples were obtained. For the first time the Gulf was systematically sampled and for the first time phytoplankton samples were collected from the surface down to 60 meters at intervals of 10 meters. All of the preceding series had consisted entirely of surface samples. The 1940 cruise (Figure 31), from October through December, was primarily concerned with the geology of the Gulf, but at twenty-two stations was phytoplankton collected in a somewhat limited area. At ten of these stations surface samples only were obtained.

The methods of collecting and enumerating the organisms were similar to those employed on the previous expeditions to the Gulf. Five liters of water, collected with the Allen closing bottle, were filtered through a net of No. 25 bolting silk and the concentrated sample preserved with a small quantity of formalin. The phytoplankton content of these concentrated samples was later determined by enumeration of the organisms in an aliquot portion in a Sedgwick-Rafter counting chamber.

A phytoplankton population is a crop in three dimensions and should be appraised from a number of samples varying in position in both horizontal vertical directions. Often when it is not possible, for one reason or another, to sample subsurface depths, the size of the population at the surface is used as an index of the total population in a water column and in this manner valuable information may be gained. Behind this latter procedure is the assumption, usually implicit, that the total population is proportional to the surface population. The use of this assumption is justified when the opportunity for more thorough sampling is lacking as was the case in the Gulf cruises prior to the 1939 cruise of the "E. W. Scripps." A difficulty arises, however, when these results, based on surface samples alone, are to be compared with the results of the 1939 and 1940 cruises when subsurface depths were sampled.

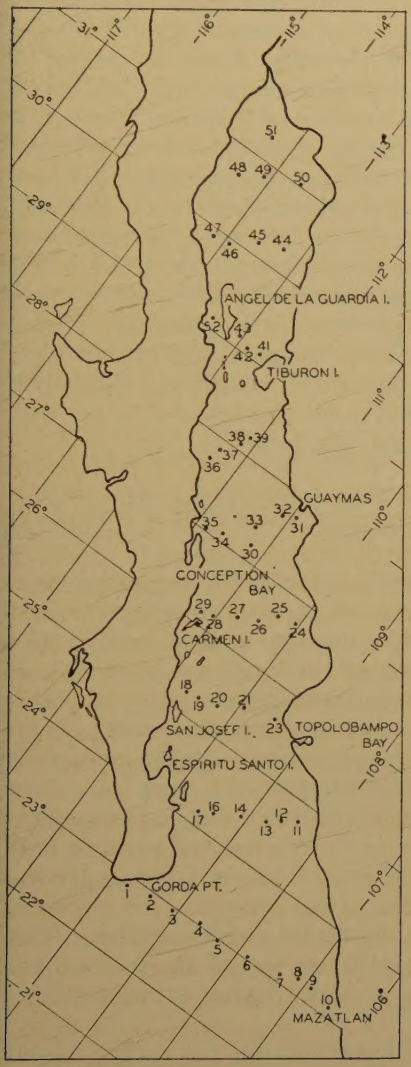

Figure 30. Location of stations at which phytoplankton samples were collected on the *E. W. Scripps* Cruise VII in 1939.

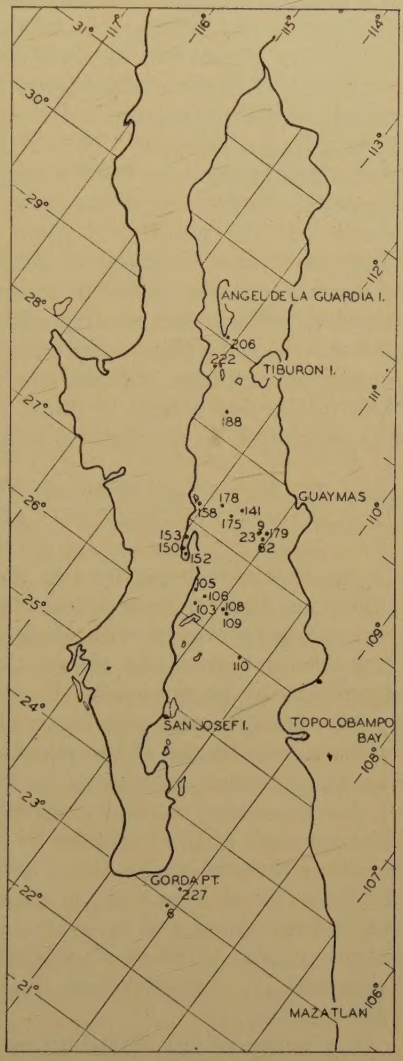

Figure 31. Location of stations at which phytoplankton samples were collected during the *E. W. Scripps* Cruise XVI in 1940.

A logical expression for the size of the total standing crop of phytoplankton is the number of plant cells under a certain area of the sea surface (cf. Bigelow et al., 1941) but this number obviously cannot be compared with the surface population of a previous cruise. A better statistic for this purpose is the mean number of cells per liter in the water column sampled. In practice the depth sampled is usually less than 100 meters, as by far the major portion of the population is found above this depth, and in the waters off Southern California it has been found that the number of plant cells below 60 meters is usually so slight that the time spent in sampling depths greater than this might be more profitably spent in increasing the number of water columns sampled. The mean number of cells per liter for this 60 meter column of water is generally of the same order of magnitude as the surface population and one will not err too greatly in comparing the two if their distinction is kept in mind. This mean number of cells per liter was calculated as a weighted mean from the formula:

$$M = \frac{\frac{1}{2}(N_0 + N_{60}) + N_{10} + N_{20} + N_{30} + N_{40} + N_{50}}{6}$$, where N_z is the number of cells per liter at the depth indicated by the subscript z.

It is not to be expected that the data obtained on these two cruises, even when combined with those of the previous cruises, will yield a complete picture of the phytoplankton of the Gulf of California. It should hardly be necessary to point out that a large number of observations are required to achieve any valid understanding of the distribution and behavior of the plankton in any region. In the laboratory one variable can be isolated by keeping constant the more important of the remaining variables, and from a relatively few measurements the responses of the organism to changes in this isolated variable may be approximated under these artificial conditions, but when studying the behavior of organisms in their natural environment, the variables can be segregated and evaluated only by statistical procedures. This entails a large number of observations or, as one of the authors has expressed it, "observations having a high degree of continuity" (Allen, 1934).

In a study of the distribution of phytoplankton the primary complicating factors are the cloud-like distribution of the plankton and the transport of these "clouds" by the water currents. At any one spot both the magnitude of the quantity and the specific composition may change completely within a few hours. Thus, at noon on December 27 at station 179, an anchor station of the 1940 cruise, there was a mean population of 2,000 diatoms per liter with *Pseudoeunotia*

doliolus and *Guinardia flaccida* making up almost half of this number; at 7:30 the next morning a second series of samples revealed a jump in numbers of diatoms to 161,400 cells per liter with *Bacteriastrum* sp. and *Chaetoceros* sp. constituting over 90 per cent of this population. The need of both continuity of observations and a knowledge of the water movements is very great. In the Gulf of California the latter is of particular importance due to the presence of a standing internal wave which causes complex water movements, both horizontal and vertical of the upper water layers.

The foregoing remarks should not, however, be taken to imply in any way that series of limited numbers of samples have little or no value. Certainly they can and do add much to our knowledge of the region under consideration and though they do not in themselves lead to complete solutions, they point the way to such solutions.

REGIONAL DISTRIBUTION OF PHYTOPLANKTON

Allen (1937) and Cupp and Allen (1938) divided the Gulf into three geographical regions for convenience in comparing abundances of diatoms. The region south of 25° N. latitude was designated as the "southern section," the region between 25° N. and 27° N. latitudes as the "middle section," and the Gulf north of 27° N. latitude as the "northern section." In the present paper the "inner" shallow region of the Gulf north of 29° N. latitude is distinguished from the "outer" Gulf region south of this latitude. The previous divisions have been retained for the outer Gulf as these divisions correspond to the regions between the antinodes of the standing internal wave and, consequently, the water masses of these three regions are more or less segregated from one another.

TABLE I—PHYTOPLANKTON, 1939: MEAN NUMBER PER LITER

Station	Diatoms: Good Condition	Diatoms: Poor Condition	Total	Dinoflagellates	Ast. japonica
1	38200	2600	40800	40	67
2	3000	300	3300	20	
3	1200	100	1400	20	
4	4300	300	4600	40	
5	31800	2100	34000	80	
6	30300	2900	32200	20	
7	20900	2200	23000	60	
8	5900	1300	7200	660	
9	13500	2000	15600	80	
10	39100	8900	48000	840	
11	2300	300	2600	500	13
12	1400	300	1600	150	
13	25300	4600	29900	300	
14	30500	5800	36300	200	
16	20600	6400	27000	60	
17	1000	500	1600	80	
18	395500	47200	442700	100	1000
19	713000	117400	830400	200	1700
20	175700	66200	241900	170	250
21	1300	700	2000	90	
23	2600	1100	3700	20	
24	400	100	500	60	
25	300	200	500	60	
26	2400	500	2900	130	
27	1200	400	1600	100	
28	1000	300	1300	150	
29	4500	200	4600	130	
30	200	200	400	60	
31	1097000	86000	1183000	30	1028000
32	89900	155800	245700	220	156000
33	800	3700	4500	120	2100
34	1600	2000	3500	90	2000
35	73600	14200	87800	150	15200
36	3100	6400	9600	110	3200
37	60100	20500	80600	80	6500
38	6600	2300	8900	40	520
39	47600	9800	57500	140	4700
41	25600	1700	27300	1600	
42	10600	6600	17200	53600	
43	88900	35400	124300	36100	170
44	11700	4600	16300	33700	
45	97400	20800	118200	35300	
46	17500	14600	32100	34300	
47	21200	10800	32000	52900	
48	27900	5900	33800	29000	
49	1600	800	2400	46900	
50	79300	158400	237700	600	
51	4700	1600	6300	203400	
52	368200	60000	428200	19700	2900

TABLE I (cont.)

Station	*Nitz. seriata*	*Plank. sol*	*Pseud. doliolus*	*Rhiz. alata*	*Rhiz. bergonii*	*Thal. longissima*	*Gon. catanella*
1	11900	110	150	47	100	280	
2	710	73	50	5	32	37	
3	180	55	17	10	8	40	
4	190	200	370	37	15	150	
5	23100	540	200	40	500	420	
6	13800	420	1100	200	210	3100	
7	11200	180	380	37	120	520	
8	1200	1400	50	100	85	980	
9	1000	110	50	160	43	140	
10	5300	140	17	120	92	120	
11	510	77	33	58	10	1300	
12	600	100	83	68		320	
13	6200	770	1600	830	140	15600	
14	7200	410	580	510	70	13900	
16	3200	120	900	140		3800	
17	60	25	67	28	2	160	
18	6400	210	83	160		340	
19	9500	300	250			180	
20	330	260	1100	27		130	
21	300	25	180			5	
23	13	7	8			3	
24	47	8	110			3	
25	45	8					
26	270	20	100		2	7	
27	360	22	73			3	
28	130	7	130	2		3	
29	98	15	100				
30	8	100	20			12	
31	8700	37	170				
32	3400	7	530	33			
33	420		33	7		20	
34	330	13	100				
35	5800	27	170			7	
36	3600	40	380	7			
37	5400	7	180				
38	780	17	82				
39	6400	13	100				
41	1200		7	13		7	1600
42	330		240				53400
43	330		980				35900
44	25		25				33700
45	5300		2100				35000
46	1700	7	1600				33700
47	1400		1600			33	52200
48	1300		2100				27600
49	200						45300
50	73100		13		34		390
51	870		33				201300
52	5800		330				19700

TABLE I (cont.)

Station	*Ch. atlanticus*	*Ch. compressus*	*Ch. debilis*	*Ch. peruvianus*	*Ch. radicans*	*Ch. vanheurckii*	*Cosc. wailsii*
1	4500	950	1200	340	1500	550	
2	190	13		25	43		
3	210					47	
4	1100			53			
5	4200			250	17		
6	2200		50	90	470		
7	1400	33	47	140	1900	710	
8	350	220	200	43	17	100	
9	67	130	940	27			
10	150	3200	280	63			
11	73			48			
12	10			33	55	33	
13	170	50	100	340	100	180	
14	520		120	160	1100	220	
16	830	450	330	50	6200	400	
17	73	50		7	50	100	
18	150	14300	1600	7	139000	191000	
19	1900	25000	11200		27100	265000	
20	310	14000	1600	7	8600	87000	
21	95	17	270		33	130	30
23	7	17			42	33	3100
24	28					33	110
25	15					33	310
26	28	33	200			960	25
27	13		170			83	55
28		50	60			42	38
29			210		3500	280	20
30							57
31		6800	43500		11600	15400	
32	20	14000	5100		7500	14900	
33		83	250				
34	55	470	620		180	67	
35	290	2200	12000		9600	49000	
36	96	3700	3400		4600	4600	
37		3700	5600		8700	6700	
38	63	450	1200		1800	500	
39	33	7600	15900		6500	4100	
41		33	2500		2100	6200	
42		13	3500		2800	650	33
43	36	850	17000		46500	9200	17
44			4600		2300	980	
45		580	32900		6500	24500	
46	50	1100	6700		1900	1300	
47		100	5900		2600	1500	
48	180	170	530		14700	2000	
49			750				
50			117000				
51			3400				
52		24700	84500		128000	47700	

TABLE II—PHYTOPLANKTON. 1940

Station	Diatoms			Dinoflagellates
	Good Condition	Poor Condition	Total	
6	700	1100	1800	
9	900	700	1600	10
23	400	200	600	13
62	5300	200	5500	7
103*	31600	21200	53800	
105*	52400	12800	65200	
106*	23200	15400	38600	
108*	18900	2900	21800	
109*	97200	37800	135000	
110	200	100	400	
141	28400	4100	32500	
150*	29900	6100	36000	200
152*	20100	5000	25100	100
153*	98750	25600	124400	380
158*	5400	600	5900	50
175	154100	38400	192500	
178*	2300	640	2900	
179a	1700	400	2100	
179b	137400	24000	161400	
179c	107000	46800	153800	
188	1500	500	1900	
206	32000	3600	35600	
222	59300	13000	72300	
227	100	200	300	

* Designates surface sample. All other stations are represented by mean populations from zero to sixty meters depth.

SOUTHERN SECTION OF THE OUTER GULF

The previous expeditions to the Gulf had found this region to be characterized by the lowest phytoplankton populations of the entire Gulf. The only catches of any considerable size found in this region were made in 1921 off Espirtu Santo Island where catches of over 100,000 cells per liter were obtained in both April and June. A few populations of 10,000 cells per liter were also found in 1937 off Gorda Point, San Josef Island, and Mazatlan.

In 1939 two lines of stations, 1 through 17, were occupied in this region. While the populations found were not exceptional from a quantitative point of view, they were larger on the whole than had been reported from it previously. All stations showed mean populations of at least 1,000 cells per liter and at 9 of the 16 stations the mean number of cells per liter exceeded 10,000.

In 1940 only two stations were occupied in this region; station 6 (Oct. 12) and station 227 (Dec. 14). The mean diatom populations at these two stations were 1,800 and 300 cells per liter respectively.

In 1939 the plankton flora of this region, stations 1 through 17,

could readily be distinguished from that of the other regions by its predominantly oceanic character. In general, *Nitzschia seriata* was the dominant species, but true oceanic forms were consistently present and constituted a considerable portion of the phytoplankton. These oceanic forms included *Chaetoceros atlanticus*, *Ch. peruvianus*, *Thalassiothrix longissima*, *Planktoniella sol*, *Rhizosolenia alata*, *Rh. bergonii*, *Rh. calcar-avis*, and *Rh. imbricata* var. *shrubsolei*. In the other sections of the Gulf these diatoms were either completely absent or were found only in a few scattered instances.

At stations 13 and 14 *Thalassiothrix longissima* was the dominant species and occurred in numbers up to 30,000 cells per liter to constitute more than half of the phytoplankton populations of these stations. This is interesting because a microscopic examination of the diatomaceous mud at the bottom of the Gulf revealed great quantities of the broken frustules of this diatom. It is one of the three forms that make up the main portion of the identifiable diatom frustules found in the Gulf sediments. The other two forms are *Coscinodiscus* sp. and *Pseudoeunotia doliolus*. In this respect it is of interest to note that *P. doliolus* was also present in considerable numbers and while this species was found at practically every station in the Gulf, it was most numerous in this southern portion and in the northern region.

MIDDLE SECTION OF THE OUTER GULF

This region, 25° N. latitude to 27° N. latitude, had been previously found to have diatom populations larger than those of the southern section but still relatively poor as compared with the more northerly regions of the Gulf. In 1921 half of the 18 samples taken in this region contained over 10,000 diatoms per liter and at several stations around Carmen Island populations of over 100,000 cells per liter were found. However, in the series of 1935, 1936, and 1937 the only samples of large populations came from off Topolabampa Bay where a catch of over 500,000 cells per liter was obtained in 1937 and from Conception Bay where populations over 10,000 cells per liter were found in both 1936 and 1937.

In 1939 huge catches of diatoms were made in the southwestern portion of this region. Stations 18 and 19 had mean populations of 442,700 and 830,400 cells per liter respectively, and station 20 had a mean population of over 200,000 cells per liter. However, the populations at the other stations of the regions were all under 5,000 cells per liter so that with the exception of the extremely large abundances in the southwestern corner, the phytoplankton of this region was the poorest found in the Gulf in 1939.

The large phytoplankton assemblages at stations 18, 19, and 20 were composed largely of neritic *Chaetoceros* species,—*Ch. radicans*, *Ch. vanheurckii*, *Ch. socialis*, *Ch. compressus*, and *Ch. curvisetus*. These neritic *Chaetoceros* species were common throughout the Gulf and usually made up the major part of the larger populations. The phytoplankton of the remaining stations of this region was clearly distinguished from that of the rest of the Gulf by the presence of moderately large numbers of a *Coscinodiscus* form either identical with or a variety of *Coscinodiscus wailsii* Gran and Angst.*

With the exception of stations 42 and 43, this diatom was not found outside of this middle region. The absence of this form at stations 18, 19, and 20 and the presence at these stations of *Asterionella japonica*, the characteristic diatom of the adjacent northern section, suggests strongly that the upper water mass at these three stations had drifted into this middle region from the adjacent northern section.

In 1940 eight surface samples were taken in this region. Six of these had populations greater than 10,000 cells per liter and one, station 153, at the mouth of Conception Bay, had a population of over 100,000 cells per liter. *Hemiaulus hauckii*, *Nitzschia seriata*, and the usually neritic *Chaetoceros* species dominated these samples. *Coscinodiscus wailsii* was not found at this time.

NORTHERN SECTION OF THE OUTER GULF

This portion of the outer Gulf, from 27° N. latitude to 29° N. latitude, includes the Guaymas area in which catches of over three million cells per liter had been made in 1937. Populations of the same size were again found here in 1939. In 1937 the main constituents of this population were *Chaetoceros radicans*, *Ch. compressus*, *Ch. debilis*, *Asterionella japonica*, and *Skeletonema costatum*. In 1939 the samples from this area, stations 31 and 32, were almost a pure culture of *Asterionella japonica*. Station 31 for example had a surface population of four million diatoms per liter, of which 3,600,000 were *Asterionella japonica*. In 1939 this species was almost entirely confined to this northern section of the outer Gulf and served to distinguish the flora of this region from that of the rest of the Gulf.

In 1939, of the ten stations in this area, five had mean populations of over 50,000 cells per liter. The phytoplankton of this region was distinctly more abundant than that of the more southerly regions.

* This diatom was identical to Gran and Angst's *Coscindodiscus wailsii* (Gran and Angst, 1931) except for the beginning of a rosette at the center, irregularly placed spinulae on the valve surface, and two small thickened processes on the valve flange at an angle of about 120° apart.

Station 30, the poorest of the section, evidently belonged to the adjacent middle section as *Coscinodiscus wailsii* was found here, whereas *Asterionella japonica* was missing.

In 1940 two stations, 175 and 179, of the ten occupied in this region had populations of over 100,000 cells per liter and two others, stations 141 and 222, had populations of over 30,000 cells per liter. The dominant species were neritic *Chaetoceros* species and *Bacteriastrum* species. *Asterionella japonica* was not found, but at station 188 a few species of *Coscinodiscus wailsii* were recorded.

INNER GULF REGION

In 1921 large diatom populations were found around Angel de la Guardia and Tiburon Islands and in the region to the north of these islands. In 1937 the region to the north of the islands was found to be very poor in phytoplankton, but exceptionally large diatom populations were found around the islands. From these series some suggestion was obtained that the area off the north end of Angel de la Guardia Island and the area off the southeastern portion of Tiburon Island were especially productive.

In 1939 these two areas off the islands were not visited, but in the rest of the inner Gulf region a completely different picture was obtained due to the presence of enormous numbers of the dinoflagellate, *Goniaulax catanella* (Whedon and Kofoid) or some species closely resembling it. Stations 41 and 50 were the only stations of this region that did not have large populations of this organism.

Diatoms were also present in large numbers in 1939. Ten of the twelve stations of this region had mean diatom populations of over 15,000 cells per liter and of these ten the mean populations of stations 43, 50, and 52 were over 100,000 diatoms per liter.

VERTICAL DISTRIBUTION

Our data are too few and our knowledge of the hydrographical conditions too vague to permit any serious attack on the problems of the vertical distribution of the phytoplankton,—especially in view of the fact that at a depth of 60 meters periodical vertical displacements of the order of 30 meters would be expected to accompany the internal wave.

In 1939 the depths of maximum populations of diatoms in good condition were distributed as follows:

0 meters — 9 stations
10 meters — 12 stations
20 meters — 9 stations
30 meters — 5 stations
40 meters — 6 stations
50 meters — 4 stations
60 meters — 5 stations.

In counting the organisms a distinction was made between the cells in good condition and those in poor condition (frustules lacking chromatophores or obviously disorganized) as the percentages of these two often aid in interpreting the results of the counts. It is interesting in this respect to note that there was no correlation between the percentage of cells in poor condition and the depth of maximum population. Nor was there any definite relation between the depth of the maximum number of cells in good condition and the depth of the maximum number of cells in poor condition—the maximum number of cells in poor condition being found as often above as below the depth of the maximum number of cells in good condition—which is in agreement with the hypothesis that the density of the cell protoplasm is close to or equal to that of sea water.

TABLE III—Vertical Distribution of Diatoms at Deep Stations, 1940

Station	*Depth in Meters*	*Good Condition*	*Poor Condition*	*Total*
9	0	480	1600	2080
	10	0	560	560
	20	20	460	480
	30	520	2000	2520
	40	1760	120	1880
	50	200	100	300
	60	60	40	100
	100	100	110	210
	300	0	65	65
	500	0	15	15
	750	0	50	50
	1250	0	5	5
	1500	5	0	5
	1620	0	10	10
23	0	140	80	220
	10	0	220	220
	20	20	160	180
	30	120	360	480
	40	760	60	820
	50	1720	80	1800
	60	0	120	120
	500	0	10	10
	750	0	40	40

TABLE III (Cont.)

Station	Depth in Meters	Good Condition	Poor Condition	Total
62	0	11520	320	11840
	10	6240	80	6320
	20	12000	440	12440
	30	2880	160	3040
	40	1440	280	1720
	50	3600	40	3640
	60	320	260	580
	500	0	40	40
	1525	0	10	10
110	0	1840	160	2000
	10	0	120	120
	20	100	40	140
	30	140	40	180
	40	140	140	280
	50	180	100	280
	60	40	220	260
	500	20	20	40
	1000	0	160	160
	1260	0	50	50
	1510	0	25	25
141	0	38400	6240	44640
	10	30400	3840	34240
	20	48160	15160	63320
	30	41760	10560	52320
	40	25440	10080	35520
	50	5440	3120	8560
	60	640	480	1120
	300	60	380	440
	500	240	350	590
	600	20	110	130
	760	70	40	110
	1000	70	170	240
	1740	10	100	110
175	0	336600	34800	371400
	10	178800	39000	217800
	20	293400	58200	351600
	30	181200	57000	238200
	40	79800	21200	101000
	50	20000	6560	26560
	60	7200	7520	14720
	300	240	820	1060
	500	0	400	400
	600	320	400	720
	750	220	750	970
	900	5	45	50
	1000	400	3740	4140
	1710	520	6800	7320
	1850	28	532	560
	1780	0	196	196
	1880	252	1540	1792

TABLE III (Cont.)

Station	Depth in Meters	Good Condition	Poor Condition	Total
179	0	333600	45600	379200
	10	313200	51000	364200
	20	270600	40200	310800
	30	62600	17200	79800
	40	9520	5040	14560
	50	1720	2080	3800
	60	320	1000	1320
	775	200	1520	1720
	860	240	640	880
	1020	0	280	280
	1200	0	1360	1360
	1020	40	100	140
188	0	320	140	460
	10	540	540	1080
	20	920	340	1260
	30	2360	440	2800
	40	1520	280	1800
	50	1400	540	1940
	60	3760	960	4720
	300	0	3560	3560
	480	222	4662	4884
	530	110	1375	1485
	580	0	495	495
	630	55	1705	1760
	670	330	3245	3575
	750	0	15000	15000
227	0	0	200	200
	10	160	240	400
	20	320	680	1000
	30	60	100	160
	40	60	20	80
	50	20	100	120
	60	80	20	100
	1000	20	20	40
	2050	60	140	200

Nine of the 1940 phytoplankton stations are of special interest in that at these stations samples were taken down to great depths; at stations 188 and 23 samples were taken down to 750 meters, and at stations 9, 62, 110, 141, 175, 179, and 188 down to depths below 1000 meters. As would be expected the number of empty frustules greatly exceeded the number of living cells at depths below 60 meters, and there was usually a sharp decrease in the number of total frustules between this depth and 300 meters, followed by a more gradual decrease below 300 meters, so that at 750 meters only a few hundred, or at the most one or two thousand, cells per liter were found. The one exception to this was station 188 where 15,000 frustules were

found at 750 meters (the greatest depth sampled) whereas populations of only a few thousand cells per liter were found in the upper layers.

The specific composition changed considerably with depth. The majority of the weakly silicified, neritic diatoms such as *Chaetoceros radicans* and *Bacteriastrum* species disappeared between 60 and 300 meters and at 1000 meters very few representatives of these diatoms were found. The heavily silicified forms which were rarely present in large quantities in the surface layers increased, therefore, relatively with depth so that below 1000 meters the empty frustules of *Coscinodiscus*, *Thalassiothrix*, *Thallassionema*, *Pseudoeunotia*, etc. completely dominated the small residue of the diatom population.

The lists of species recorded for diatoms (table IV) and dinoflagellates (table V) are essentially the same as those recorded in other reports (e. g. Cupp and Allen, 1938), no significant differences being discernible.

TABLE IV—Diatoms Found in the Gulf of California in 1939 and 1940

Actinoptychus undulatus (Bail.) Ralfs	**Asterolampra marylandica* Ehr.
Asteromphalus heptactis (Breb.) Ralfs	*Asterionella japonica* Cl.
Bacteriastrum sp.	*Biddulphia* sp.
Chaetoceros affinis Laud.	*Ch. atlanticus* Cl.
Ch. compressus Laud.	*Ch. curvisetus* Cl.
Ch. debilis Cl.	**Ch. diadema* (Ehr.) Gran & Angst
Ch. didymus Ehr.	*Ch. lacinisosus* Schütt
Ch. lorenzianum Grun.	**Ch. messanensis* Castr.
**Ch. pendulum* Karst.	*Ch. peruvianus* Brightw.
Ch. radicans Schütt	*Ch. socialis* Laud.
**Ch. vanheurckii* Gran	*Chaetoceros* sp.
**Climacodium fraunfeldianum* Grun.	
Corethron criophilum Castr.	*Cor. hystrix* Cl.
**Coscinodiscus wailesii* (?) Gran & Angst	*Coscinodiscus* sp.
**Coscinosira polychorda* Gran	
**Dactyliosolen antarcticus* Cast.	*Dac. mediterranea* Perag.
Ditylum brightwellii (West) Grun.	*Eucampia zoodiacus* Ehr.
**Fragilaria* sp.	**Gossleriella tropica* Schütt
Guinardia flaccida (Castr.) Perag.	*Hemialus hauckii* Grun.
Lauderia borealis Gran	*Leptocylindricus danicus* Cl.
Lithodesmium undulatum Ehr.	**Licmophera lyngbyei* (Kutz.) Grun.
**Melosira* sp.	
**Navicula membranacea* Cl.	**Nav. pelagica* Cl.
Navicula sp.	
Nitzschia longissima (Breb.) Ralfs	*Nitz. seriata* Cl.
Nitz. pungens Cl.	

* Species not previously reported from the Gulf of California.

TABLE IV (cont.)

**Paralia sulcata* (Ehr.) Cl.	*Planktoniella sol* (Wall.) Schütt
Pleurosigma sp.	*Pseudoeunotia doliolus* (Wall.) Schütt
Rhizosolenia acuminata (Perag.) Gran	*Rhiz. alata* Brightw.
Rhiz. bergonii Perag.	*Rhiz. calcar-avis* Schultze
Rhiz. castracanei Perag.	*Rhiz. delicatula* Cl.
Rhiz. fragilissima Berg.	*Rhiz. imbricata* Brightw.
**Rhiz. robusta* Norm.	*Rhiz. hebetata* f. *semispina* (Hensen) Gran
Rhiz. setigera Brightw.	*Rhiz. stolterfothii* Perag.
Schroederella delicatula (Perag.) Pav.	*Skeletonema costatum* (Grev.) Cl.
**Stephanopyxis palmeriana* (Grev.) Grun.	**St. turris* (Grev. & Arn.) Ralfs
Surirella sp.	
Thalassiosira decipiens (Grun.) Jorg.	**Thal. gravida* Cl.
**Thal. pacifica* Gran & Angst	*Thal. rotula* Meun.
**Thal. subtilis* (Ostf.) Gran	
Thalassiothrix frauenfeldii Grun.	*Th. hetermorpha* Karst.
Th. longissima Cl. & Grun.	
Thalassionema nitzschoides Grun.	**Tropidoneis* sp.

*Species not previously reported from the Gulf of California.

TABLE V—Dinoflagellates Found in the Gulf of California in 1939 and 1940

Ceratium candelabrum (Ehr.) Stein	*Cer. furca* (Ehr.) Clap. et Lachm.
Cer. fusus (Ehr.) Duj.	*Cer. macroceros* (Ehr.) Cl.
Cer. kofoidii Jörg.	*Cer. pentagonum* Gourret
Cer. tripos (O. F. M.) Nitzsch	
Dinophysis caudata Saville-Kent	*Din. ellipsoides* Kof.
Goniaulax catanella Whedon & Kofoid	*Gon. polyedra* Stein
Goniaulax sp.	
Noctiluca miliaris Sur	
Peridinium crassipes Kof.	*Per. depressum* Bail.
Per. divergens Ehr.	*Per. oceanicum* Vanhöffen
Per. orbiculare Paulsen	*Per. ovum* Schiller
Per. pyriforme Paulsen	*Per. pellucidum* (Berg.) Schütt
Per. steinii Jorg.	*Peridinium* sp.
Phalacroma rotundatum (Clap. et Lachrm.) Kof. & Mich.	*Podolampas palmipes* Stein
Prorocentrum micans Ehr.	

SEASONAL VARIATION

The productivity of the Gulf of California, so far as this is represented by the size of the phytoplankton populations, appears to be relatively great, but the data are far too deficient in continuity, both temporal and spatial, to permit any definite conclusions. Populations of over 100,000 cells per liter have been found at various stations throughout the Gulf in every season it has been sampled, but whether

this would have been found to be the case by a continuous investigation extending over this period of the year, from November through June, is not known. Since three of the six cruises occurred in February and March, our knowledge of this period is more complete. In two of these three cruises huge populations were found, and it appears probable that this is the period of larger populations (a little earlier than in the Gulf of Santa Catalina).

The evidence for the annual phytoplankton cycle is largely indirect. Bottom cores from the Gulf show alternating light and dark bands which when examined under the microscope prove to be layers of relatively pure diatomaceous material alternating with layers of less purely diatomaceous detritus. These layers have been interpreted as annual layers corresponding to seasonal changes in diatom populations (Sverdrup and Staff, 1940) and if so indicate large seasonal differences in phytoplankton production.* In addition the waters are reported to be clear and blue in late summer but are discolored with phytoplankton growth over large areas in late winter and spring.

In the beginning of November strong northwesterly winds set in which must result in upwelling and replenishment of the nutrients in the surface layers. At the same time vertical circulation is brought about in the shallower upper region of the Gulf by the cooling of the surface waters with resulting lowering of the stability. These conditions should result in greatly accelerated phytoplankton production. In early summer the northwesterly winds die out; the surface waters become heated and stratified; and the phytoplankton should rapidly exhaust the supply of nutrients and diminish in quantity. This picture is partly confirmed by the fact that the largest populations have been found in March.

GENERAL CHARACTERISTICS OF THE GULF OF CALIFORNIA

The Gulf of California is a sloping trough, roughly V shaped in cross section, approximately 700 miles long and 60 miles wide. At the entrance its waters communicate freely with those of the Pacific Ocean down to a depth of more than 3,000 meters. The bottom slopes upward toward the Gulf's head and is irregular with several deeper basins present. About 29° N. latitude the Gulf becomes quite shallow and a group of islands rise above the surface. Beyond this point there are two deeper basins on each side of Angel de la Guardia Island but from them northward the depth is less than 200 meters.

* It should be borne in mind, however, that the diatoms whose frustules make up the diatomaceous deposits are not the same as those that make up the bulk of the phytoplankton pulses.

The temperature and salinity of the deep water in the outer Gulf show that this water is of the same type as exists over large areas in the Central Pacific. The general water circulation is characterized by an inflow of deep water and an outflow of surface water, and as a consequence of this circulation deep water, rich in plant nutrients, is brought to the euphotic zone where the nutrients can be utilized by the phytoplankton. The main factor effecting this circulation in the outer Gulf is the wind, which from late fall to summer blows parallel to and out of the Gulf, bringing about upwelling and an outward transport of surface water.

In the upper Gulf, i. e. in the region of and north of Angel de la Guardia and Tiburon Islands, there are two basins with sill depths of less than 200 fathoms in which temperature, salinity, and oxygen curves (Sverdrup, 1940) show that basin conditions exist. In winter, at the same time the northwesterly winds are bringing about upwelling in the outer Gulf, the surface temperature is decreased by cooling, and convection currents develop in these basins and in the shallow region to the north of the islands.

Thus in both inner and outer regions of the Gulf the hydrographical features are conducive to high productivity. These two conditions, upwelling in the outer basin and convection in the inner basin, can fully account for the fertility of the Gulf without the necessity of considering the effect of the Colorado River which empties into the head of the Gulf. That vertical oceanic circulation can maintain an enormous amount of marine life in the absence of local terrestrial drainage is clearly shown by the great productivity of the waters off the West Coast of South America which is one of the most arid coastal regions in the world.

At the time of the 1939 cruise salinity, temperature, oxygen, and density curves showed upward displacements suggestive of upwelling at stations 23, 39, and, to a lesser extent, 31. However, the hydrographical conditions in the outer Gulf in 1939 suggest the existence of a standing internal wave. Such a wave could give rise to similar displacements or could reduce the displacements due to upwelling, and caution must be exercised in utilizing such profiles to locate the centers of active upwelling.

The distribution of mass at the time of the 1939 cruise showed alternating upward and downward displacements of the isopycnal surfaces, and the dynamic computations revealed a sequence of separate highs and lows of the isobaric surfaces. This suggested the existence in the outer Gulf of a standing internal wave having three nodes (Sverdrup, 1940). The distribution of the mean diameter of the par-

ticles of the bottom sediments was found to agree with the presence of such a wave (Revelle, 1940) and suggested that this wave is a permanent feature of the Gulf. A theoretical treatment by Munk (1941) further showed that the postulated characteristics of the wave were compatible with the physical features of the Gulf. Additional confirmation for the existence of this wave now appears in the distribution of the phytoplankton.

The presence of such a standing internal wave with three nodes would be associated with three circulation cells in the outer Gulf whose boundaries would be located at the antinodes and a fourth cell comprising the area north of the island region where the wave terminates. This internal wave would set up horizontal cellular currents having maximum velocities of the order of 50 cm/sec but having zero velocity at the antinodes or cell boundaries. The direction of these currents in the middle cell would be opposite to those in the southern and northern cells and these directions would be reversed every half period, i. e. about 3.5 days for a first order wave or 7 days for a second order wave. In the case of waves with periods as great as these, the deflecting force of the earth's rotation would modify the direction of these currents so as to bring about a circular flow instead of a simple oscillation in one direction. This cellular circulation would act as a barrier to intercellular flow and would tend to preserve for a time any differences in the plankton flora of the cells.

In addition to this intracellular circulation there will be tendencies for intercellular circulation due to alterations in the distribution of mass brought about by the tangential stress of the wind and thermohaline changes in density. This will result in the frequent breakdown of the intercellular barriers and the transport of water masses across the antinodal regions. Any floral differences reflecting the presence of the internal wave will, therefore, be transitory in nature.

This internal wave greatly complicates the interpretation of the hydrographical data. As was mentioned previously, profiles of the physical properties of the water showed a large upward displacement at station 23. Such an upward displacement usually indicates upwelling and a consequent rich supply of nutrients in the surface layers. Yet this region was found to be extremely poor in phytoplankton. This raises the question as to whether the upward displacements of the isolines really indicated active upwelling. It is quite possible that this distribution of properties reflects the presence of the internal wave rather than upwelling. An anti-cyclonic circulation in this middle cell would be accompanied by upward displacements of the sigma-t surfaces along the shores and a downward displacement in the

center; a condition which would simulate upwelling. This distribution of density would be reversed in the next half period when the anti-cyclonic circulation changed to a cyclonic one and any upwelling in this area would then be masked. Since the circulation in the adjacent cells is opposite in direction to that in the middle cell, the latter appears to be the case off Guaymas where a slight upward displacement of the isopycnals was accompanied by an extremely large diatom population.

On the other hand, it is equally possible, in the absence of any data and neglecting any intrinsic factors preventing the diatoms from responding to an increase in nutrients, to attribute the low populations of the middle region to zooplankton grazing. Also, other seasons of the year, or other years, may show differences in conditions.

SUMMARY

The examination of the distribution of the phytoplankton in the Gulf of California in 1939 disclosed four distinct phytoplankton types; an oceanic type at stations 1 through 17, a *Coscinodiscus wailsii* type at stations 21 through 30, an *Asterionella japonica* type at stations 31 through 39, and a *Goniaulax catanella* type at stations 41 through 52. The distribution of these types corresponds exactly to the locations of the circulation cells which would be predicted in the presence of a standing internal wave as postulated by Sverdrup and constitutes confirmative evidence for the existence of this wave.

In agreement with the results of previous cruises to the Gulf, the phytoplankton populations of the northern sections of the Gulf were found to be larger on the whole than those from the southern section. This indicates that upwelling is most active in the northern section of the outer Gulf (the Guaymas area) where slight upward displacements of the isopycnal surfaces were found in spite of an opposing tendency of the distribution of mass accompanying the internal wave.

Indirect evidence indicates that the seasonal phytoplankton cycle is dependent upon the process of upwelling in the outer Gulf and upon convection in the upper, shallow region. These two processes are initiated in late fall when northwesterly winds bring about upwelling and when surface cooling lowers the stability of the water masses in the basins and shallow areas of the upper Gulf. At the onset of summer when the northwesterly winds die out and the surface waters become heated and stratified, these processes and the spring phytoplankton growth terminate. The fact that the largest populations have been found in March supports this picture and suggests that this is the period of maximum productivity.

Phytoplankton samples from depths greater than 300 meters were obtained in 1940 and indicate that the majority of the weakly silicified cells were dissolved above this depth and that the numbers of frustules cleansed of cell contents greatly exceeded the number of cells in apparently good condition.

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A NOTE ON SOME LABORATORY MEASUREMENTS OF THE TRANSPARENCY OF SEA WATER

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Laboratory measurements of the absorption of light by sea water have been reported for surface samples from a number of stations in the Atlantic (1, 2, 3, 4). The work of Clarke and James (4), which includes results on 12 stations, both in and off shore for wave-lengths 3650–8000 Å, is of particular interest. They find that for off-shore waters the difference between the absorption of sea water and pure water at all wave-lengths is due practically entirely to suspended materials, termed "suspensoids," which can be filtered out. For off-shore waters these suspensoids are non-selective with respect to wave-length. For in-shore waters two agents are responsible for absorption: suspensoids, responsible for the major part of the absorption, and a filter passing material. In this case both are selective in their action on light. The effect of suspended materials in lake-waters had been previously investigated by Pietenpol (5) and by James and Birge (6). Suspensoids (the term was coined by James and Birge) were found to be the major factor involved in most of the waters they studied. Filter passing material was also present which was highly selective in its absorption of light. It is the purpose of this note to describe some similar measurements on samples obtained down to 60 meters depth in the Pacific Ocean.

The essential features of the absorption apparatus are shown in Figure 32. A parallel beam of light (angular divergence 6′) *L*, 1.2

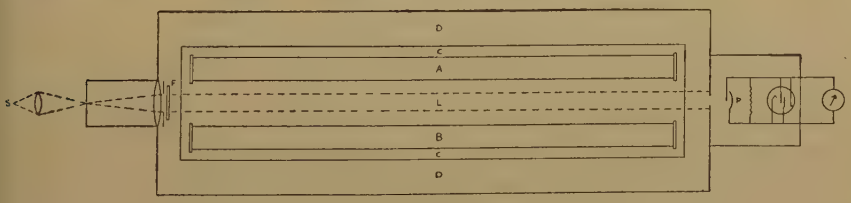

Figure 32. Absorption Apparatus.

[1] Contributions from the Scripps Institution of Oceanography, New Series No. 203.

cm in diameter, passed through a glass tube 2.5 cm in diameter and 1 meter long. The beam then passed through an aperture 0.8 cm in diameter and was incident on an RCA 917 vacuum photocell, *P*. Two identical tubes, *A* and *B*, were mounted rigidly in the parallel grooves of an optical bench which was fastened to a flat steel plate, *C*. This latter could be moved by means of a rack and pinion parallel to itself across a 10″ channel beam, *D*, so as to bring either of the tubes into the light beam. The ends of the tubes were ground perpendicular to the axes of the tubes to within 30″ and glass flats were pressed against the ends by external collars. The glass to glass contacts between the flats and the tubes were sealed with stop-cock grease. Samples of water in the tubes thus came in contact only with glass. The tubes were filled and emptied through side arms. After the tubes were in adjustment they produced no visible displacement of the light-beam, and for identical samples of water the recorded intensity of light for either tube was the same. The light source was a tungsten filament, 108 watt, 6 volt projection lamp. The photocell current was amplified by a single stage amplifier using an RCA 954 pentode acorn tube in a circuit described by Gabus and Pool (7). The steady plate current was balanced out and the full range of the galvanometer scale utilized for differences in photocell current between the two tubes. With the light intensity used the overall sensitivity was such that a one percent change in intensity corresponded to a 1 cm deflection of the galvanometer. The circuit was capable of much greater sensitivity but stability becomes an increasing problem and for the purposes of this investigation the above sensitivity is sufficient.

A Corning "Sextant Green" filter, *F*, with maximum transmission at 5400 Å was used throughout the course of the experiment, and all the data refer to light of spectral distribution produced by this filter. One tube was filled with twice distilled water from an all glass still. During a period of two months no change in absorption was detected in this sample, showing there was no contamination from the tube. The average of a large number of readings, corrected for reflection losses, gave 84.3% for the transmission of one meter of pure water relative to air. This value is in agreement with the results of James and Birge (6) if one combines their transmission values at various wave-lengths with the transmission values for the filter. (At 5400 Å James and Birge find a transmission of 97%; however the filter passes sufficient light of longer wave-lengths, which are much more strongly absorbed, to reduce this value to that stated above.)

All measurements on sea-water consisted of a comparison of the transmission of a given sample with that of pure water by shifting the

tubes back and forth, and the data are in terms of percent transmission relative to pure water. Samples were obtained from the following stations:

Station	*Location: off-shore La Jolla Cove*
A	12.7 miles
B	11.4
C	10.2
D	8.9
E	7.6
F	6.4
G	5.0
H	3.8
I	2.5
J	1.3
K	0.1

At stations A and G samples were obtained every 10 meters down to 60 meters. At the other stations only surface samples were obtained. All samples were stored in glass containers and were tested within 24 to 72 hours after collection. Samples from station A at all depths and surface samples from C and H were passed through a Berkefeld filter. All samples were well ahaken before being placed in the absorption tube. Tests on both filtered and unfiltered samples from station A at 10 meters and from station G, surface, every 24 hours for 4 days showed no significant variation with time. It is estimated that the data on relative transmissions are accurate to within 2%.

The results of the absorption measurements are shown by the curves of Figure 33. The curve for station A (unfiltered), with its

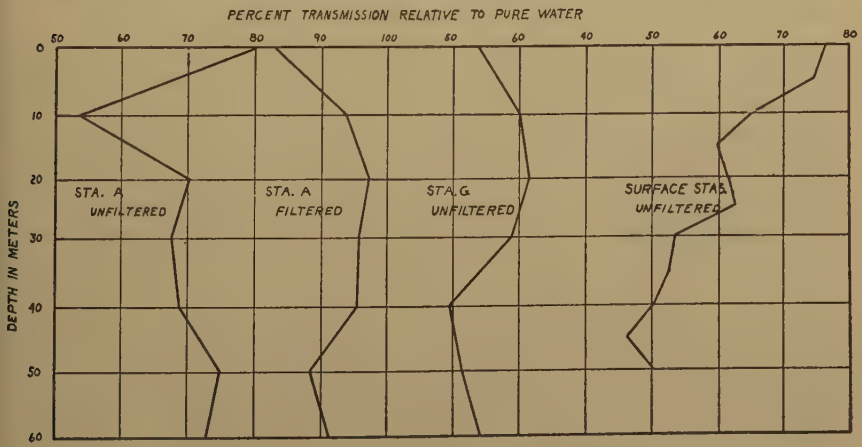

Figure 33. Curves of Relative Transmission.

pronounced minimum is of the same form as many of the curves based on transparency meter data taken in 1938 at many stations in the same general region (8). The existence of a sharp minima in transmissivity seems to be a distinguishing characteristic of the water at many stations. The curve for station G does not correspond to any of the transparency meter curves. At station A the water was shading towards the blue color characteristic of deep ocean waters; at G the water was green, characteristic of inshore waters. The curve for the surface samples, though irregular, shows the water to be increasingly turbid as we come towards shore, which is what we would expect.

The effect of filtering is shown by the filtered curve for station A. With the exception of the surface sample[1] filtering increases the relative transmission of all samples up to 90 to 95% that of pure water, whereas the unfiltered samples ranged from 50 to 80%. Two other samples were also filtered; namely, the surface samples at stations C and H. For these the relative transmission was increased from 65 to 91% and from 53 to 96% respectively. It is of particular interest to note that the sharp maximum of the unfiltered curve has completely disappeared. It is evident that the main factor involved in the absorption of light is material which is stopped by the filter. The porosity of the filter used was such that the majority of particles greater than one micron in diameter were stopped. The residual absorption of the filtered sea water may arise either from suspensoids fine enough to pass through the filter, or from dissolved material.

It may be of interest to report some subsidiary experiments made during the course of this investigation, which give some information concerning the nature of the suspended material. Tests were made on water filtered through a standard mesh used in nets for collecting plankton. These tests showed that any material taken out by the net had little if any effect on the transparency of the water. The relative transmission of the same samples when filtered through the Berkefeld filter was raised from 28 to 88%. Therefore one cannot ascribe the transparency characteristics of sea water to plankton or plankton debris which are removed by a plankton net. This is in line with results obtained with the transparency meter (8), and by others (9, 10), which have shown it is impossible to correlate transparencies with plankton densities.

Another experiment was to allow a column of sea water 35 cm high to stand undisturbed for three days. Measurements were then made on the transmission of the upper and of the lower half. The upper

[1] The result for this sample is so much out of line with the rest of the data that it is felt that this particular sample became contaminated in some way.

half was only 1% more transparent than the lower. The transmissions of these samples were 68 and 67% that of pure water. Hence there is no appreciable settling of the material responsible for the turbidity of the water. Thus suspensoids can exist in fairly definite layers and produce sharp minima of transparency such as observed at station A.

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A SUGGESTED COUNTING FRAME FOR INTERTIDAL POPULATION STUDIES

By

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Many investigators have made population studies on the intertidal region, particularly to determine the zonation of marine life in correlation with tidal levels. These workers for the most part have adopted the commonly used square meter or square foot quadrat, or fraction of same, so successfully employed in the study of terrestrial populations. In some cases, such as extensive, gently sloping beaches and flats this method has been very satisfactory. On steeply sloping shores, however, and on rocky ledges, large boulders, etc. the angle of a quadrat is so great that a square meter quadrat would often overlap several natural levels of distribution. Such a quadrat at an angle of 40° has a vertical range of 65 centimeters (7¾ inches for square foot quadrat). At 60° it has a vertical range of 86.5 centimeters (10⅜ inches for square foot quadrat), and even at 20°, a range of 32.5 centimeters (4¼ inches for square foot quadrat).

On many shores, especially those with a small range in tide, these vertical distances would cut through two or more critical levels so that the quadrat count is not a true sample for any zone, and indeed zones could easily be entirely obscured by such sampling.

Where time or conditions do not permit a complete transect to be made, and random samples are taken at different levels, the writer suggests oblong counting frames to include either one-fourth of a square meter or one square foot of surface, but constructed to be 20 centimeters by 125 centimeters in one case and 6 inches by 24 inches in the other. Such counting frames applied to sloping shores, ledges and rocks would give a better sample of any particular level, having as they do, a longer horizontal stretch to increase the chances of including widely scattered forms such as certain snails or hydroids, and would include more organisms of a common level, at the same time excluding higher and lower levels. These oblong quadrats would be wide enough to accommodate a narrow shovel or clam fork for digging into sediments. Where it is desirable to sample a greater area or dig up a greater volume of sediment, the counting frame can be applied several times in a series along the same grade to keep the sample within

the same tidal level. In this way a larger sample can be taken inside a particular zone.

The writer, having used the conventional square forms of various sizes for a number of years, has come to the conclusion that an oblong counting frame would give a more accurate sample of a particular level and a more adaptable instrument for zonation studies, especially on shores having a number of sharp vertical limits. It is the plan of the writer to use such a frame at the earliest opportunity, but he hopes that in the meantime others might make use of it.

THERMAL AND DIURNAL CHANGES IN THE VERTICAL DISTRIBUTION OF EGGS AND LARVAE OF THE PILCHARD (SARDINOPS CAERULEA)[1]

By

RALPH P. SILLIMAN

INTRODUCTION

Knowledge of the vertical distribution of pilchard eggs and larvae is of interest from two standpoints: first, in adding to the general knowledge of the early life of this species in the sea; and second, in defining the vertical range of its early stages so that regular surveys of the spawning grounds may be designed to sample all strata in which eggs and larvae are to be found. In reference to the second point it was desired if possible to relate vertical distribution to physical characteristics of the sea, so that the depth to which nets must be sent might be predicted. It was felt that the concentration of organisms at a given depth would be controlled chiefly by temperature, density, and light intensity. The analyses described in this paper have been designed to discover and define the relationships between these three variables and the vertical distribution of pilchard eggs and larvae.

METHODS

The field technique employed throughout the experiment was to make at each station successive net hauls, each sampling a layer of water below that of the preceding haul. In 1939, hauls were approximately horizontal. In 1941, however, oblique hauls were adopted to preclude the possibility of missing concentrations of larvae in the gaps between hauls. Each oblique haul extended diagonally from the top of the layer to be sampled by the next haul, to the bottom of the layer sampled by the preceding haul. The "surface" haul was made with

[1] The data on which this report is based were collected as part of a joint program of oceanographic research carried on by the United States Fish and Wildlife Service and the Scripps Institute of Oceanography. Mr. O. E. Sette, in charge of the South Pacific Investigations of the Fish and Wildlife Service, suggested the investigation of the particular problems discussed in this report, and gave much helpful advice. Sorting of the plankton collections was accomplished with the assistance of Works Progress Administration projects Nos. 10917 and 65-2-08-286.

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an open net, while for the others the net was lowered closed to the desired level, hauled for from 15 to 20 minutes, closed, and brought to the surface.

Closing nets used were identical, except for dimensions and materials, with the one described by Leavitt (1935), and the tripping device was the one figured by him in a later paper (Leavitt, 1938, fig. 2). Sizes of nets and materials used are given in the list of stations at the end of this paper.

Temperatures were taken in 1939 with reversing thermometers, and in 1941 with a bourdon-type bathythermograph modified from the one described by Spilhaus (1940).

Water flow was measured in the 1939 hauls by a meter consisting of an impellor and counter. "Stray angle" (departure of the towing wire from the vertical) was measured with a pendulum protractor.

PERIOD AND AREA COVERED

Stations were located within a radius of 120 miles of San Diego, California, and were in an area somewhat to the south and east of the areas of heaviest pilchard spawning as indicated by other surveys. Four series of hauls were made in April, 1939, and seven in April, May and June, 1941. Exact locations, dates, and depths fished are given in the list at the end of the paper.

CLASSIFICATION OF MATERIAL

Eggs and larvae were of course treated separately. Also, to take account of the fact that motility increases with size of larvae, these were divided into two categories: "large" and "small." A dividing line of 8 mm. had previously been used in other work, with which it was desired to compare the results of this analysis. Since this line did not split a mode in the length frequency distribution of larvae in the vertical serial hauls, it was retained for the present study.

Some larvae of the "small" group were lost through the meshes of the one-meter nets used. This, however, does not necessarily invalidate comparisons between numbers caught at different levels with the same net, since the loss should be a constant percentage of the total number caught.

STANDARDIZATION OF HAULS

Because of unavoidable variations in the speed of the ship and time of hauling, the amount of water strained by the net varied from haul to haul, even within a single series. These variations were discounted by putting all the hauls in each series on the basis of a standard volume

of water strained. For the 1939 hauls flow of water through the nets was measured directly by a current meter, but for the 1941 hauls, the relative amount was computed from the formula $\frac{W_1}{W_2} = \frac{\tan \angle_1}{\tan \angle_2}$, where $\frac{W_1}{W_2}$ = the ratio between the amounts of water strained for any two hauls, and $\angle_1$ and $\angle_2$ = their respective stray angles. Actual volumes of water strained averaged around 400 cubic meters for the one meter nets, and 100 cubic meters for the half-meter nets. The numbers of organisms in the hauls were multiplied by factors $\left(\frac{V_s}{V_h}\right)$, where V_s = standard volume of water and V_h = volume strained in given haul) which made the numbers the same as if the standard volume of water had been strained, and had contained the same concentration of organisms as the water actually strained.

Because of the great range in numbers of organisms (from 6 to 6000 in a single series of hauls) they could not be represented graphically on the same scale. Since the change in concentration from one series to another was not of interest, but only the changes from haul to haul within a series, the graphing difficulty was overcome by representing each vertical series by a polygon of equal area. To do this, the product of numbers of organisms by thickness of layer sampled was obtained for each haul. These products were summed for each station, and the original counts of organisms were multiplied by factors which made the sum of such products a constant for all stations. Besides overcoming the graphing difficulty, this computation placed the numbers at each level for all of the stations on a comparable basis, permitting direct comparison of catches at different levels even though they were not taken at the same station.

To avoid excessive random variability from small numbers, series containing less than 100 eggs, 50 small larvae or 5 large larvae were omitted from the analysis.

In order to construct the polygons mentioned above, it was necessary to compute the average depth for each haul. For the 1939 hauls, when the stray angle was measured two or three times during a haul, the angles were simply averaged and the depth computed from the average angle and the length of the towing wire. In 1941, when angles were measured each minute during a haul, it was possible to construct a plot of the course of each haul (Figure 34). Average depths were computed from the plots by measuring the area bounded by each one and its baseline, and dividing by the length of the baseline.

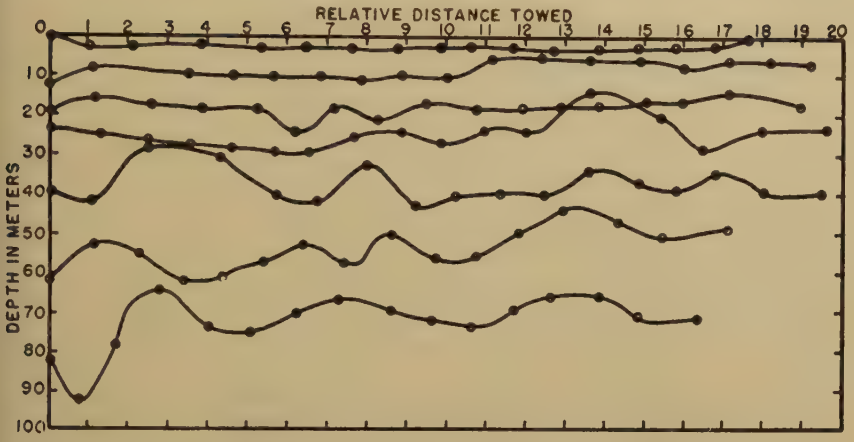

Figure 34. Course of serial hauls for a typical station (No. 2046). "Relative distance" was computed from the product of time and average tangent of angle of stray for each one-minute interval of the hauls. Depths were computed from cosine of angle of stray.

EFFECT OF TEMPERATURE

Inspection of the vertical profiles for relative numbers of eggs and larvae, together with the corresponding temperature profiles (Figure 35) indicates a possible relationship between temperature and vertical distribution. Such a relationship could arise as the result of the seeking of an optimum temperature by the larvae, and by the adult fish which lay the eggs. Since pelagic fish eggs tend to remain in water of the same specific gravity as that in which they were fertilized (Walford, 1938) the vertical distribution of eggs should tend to reflect that of the parent fish at the time of spawning. This relationship is complicated by vertical turbulence above the thermocline, which tends to disperse passive bodies like fish eggs.

Whatever the nature of the relationship between temperature and the distribution of pilchard eggs, the larvae may be expected to respond directly to changes in temperature, since they are capable of locomotion. Again the relationship is probably complicated by other factors, such as the amount of fodder organisms present.

To measure the correlation between temperature and concentrations of eggs and larvae, the relative numbers were plotted as regressions on degrees Centigrade (Figure 36). Hauls above a depth of 10 meters were omitted because of the reversals in egg and larvae profiles which often occurred in that layer. Also excluded were zero hauls below the first. Inclusion of either of these two categories of hauls would

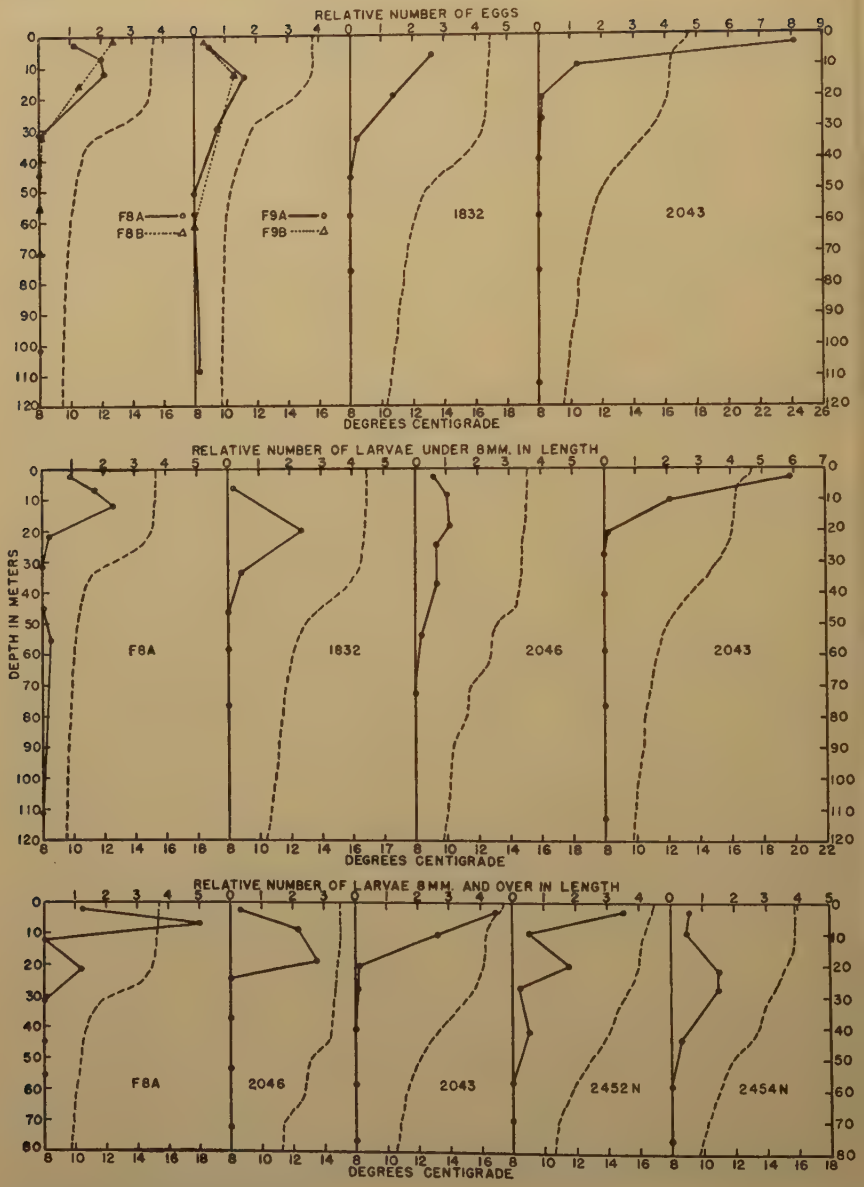

Figure 35. Realtive numbers of eggs and larvae taken at each depth. Broken line shows temperature. For the large larvae two series taken around noon have been omitted because of the effect of light. They are shown in Figure 37.

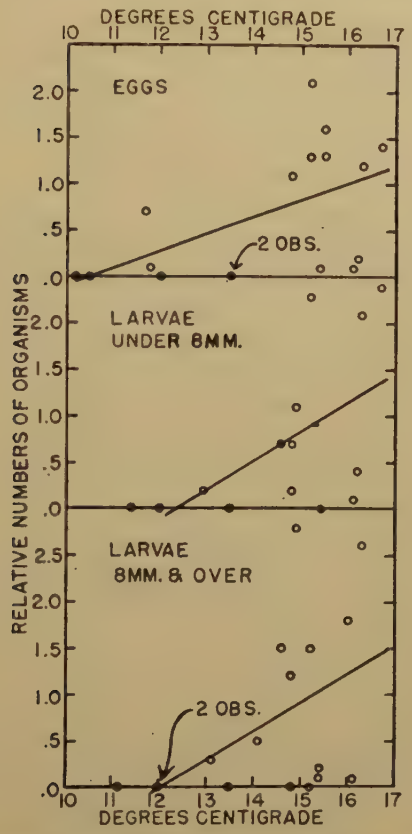

Figure 36. Regressions of numbers of eggs and larvae on temperature. Lines shown are "least squares fits."

obscure the decrease from the maximum concentrations down to zero, which is the point of chief interest in the regressions insofar as they are to be used in determining the depth of net hauls for regular surveys. Correlation coefficients were .590 for eggs, .557 for small larvae, and .548 for large larvae, corresponding to probabilities of .010, .041 and .024 of chance occurrence. Combining these probabilities by the method of Fisher (1936, p. 105) indicates that the result, as a whole, is highly significant ($P = .0009$).

Of special interest in the foregoing correlations are the intersections of the computed regression lines with their baselines, since these should indicate the temperature below which we would not expect to find eggs or larvae. The indicated minimum temperatures are 10.5°

for eggs, 12.4° for small larvae, and 12.0° for large larvae. Since the regressions for large and small larvae were found by Fisher's (1936 p. 146) t test not to differ significantly in slope, they were combined, giving an indicated minimum of 12.0° for both size categories.

DIURNAL CHANGES

To determine the extent and nature of diurnal changes in the vertical distribution of pilchard larvae, two special pairs of serial hauls were run in June, 1941, the first of each pair being occupied around midnight, and the other around noon. Due to unavoidable movements of the vessel the two members of each pair were not located at exactly the same point geographically, but approximately the same position was maintained and they were probably in water of similar characteristics.

One of the chief differences between the night and day hauls was that the former contained many more large larvae than the latter (Figure 37). This could result from any one of three causes: (1)

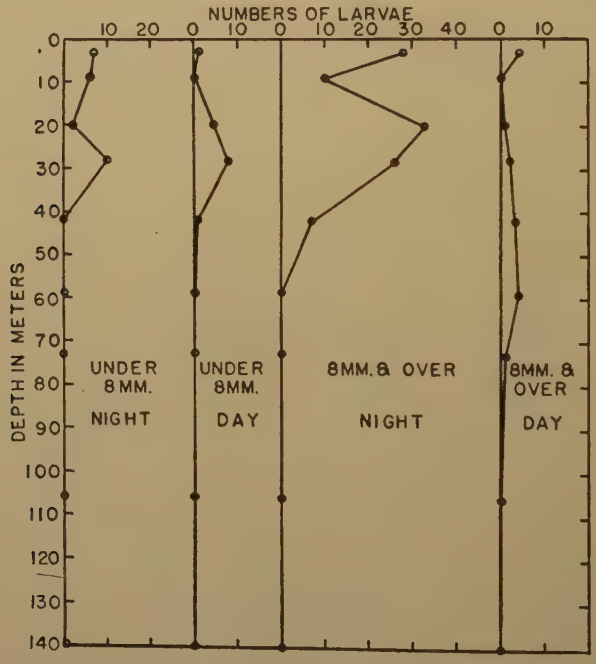

Figure 37. The combined catches of stations 2452 and 2454, comparing day and night hauls at each depth. Numbers represent actual numbers caught.

location of the day hauls in an area of lesser concentration than the night hauls, as a result of movement of the ship through the water between the two series; (2) migration of most of the larvae below the levels fished, during the daytime; (3) dodging of the net by the larvae, in the daytime.

The first explanation is a highly improbable one, since the larger catches at night have been observed many times previously, for other plankton organisms and larvae of other species of fish. Also, our regular surveys of the spawning grounds show the night catches of larvae to be consistently larger than the day.

The second explanation has been most widely entertained by others investigating vertical migrations. For instance, it was advanced by E. S. Russel (1928) to explain the larger catches of clupeoid and gobioid larvae at night, but he was later forced to abandon it when subsequent daylight hauls near the bottom (Russel, 1930) did not average larger catches than the ones in the upper layers of water. For our own data, the taking of at least two blank hauls (Figure 37) below those which contained any larvae argues against the probability of a concentration of larvae below the levels sampled.

Elimination of the first two explanations leaves only the third—dodging of the net in the daytime. Russel (1926) and Johansen (1925) admit this possibility, but doubt that fish larvae could swim fast enough for successful dodging. On this point it may be well to consider how fast it would be necessary for larvae to swim in order to get out of the way of the net. All nets towed in the conventional manner give warning of their approach by means of the piece of line which attaches them to the towing wire. In our gear this line was about 5 meters long, while our average speed of towing was around 50 cm. per second. The larvae would thus have about 10 seconds to swim out of the way. Since the maximum distance they would have to swim would be the radius of the net, or 50 cm., a swimming rate of 5 cm. per second would be sufficient if the direction of swimming were favorable. This does not seem an incredible rate for pilchard larvae of the size in question (over 8 mm. in length). Likewise it does not seem unreasonable that they should swim away from the center of disturbance, and therefore in a direction favorable to escape from the oncoming net.

In the day hauls the large larvae were not only less numerous than in the night hauls, but also appeared to be distributed in somewhat deeper levels. A greater *relative* number of larvae at the deeper levels in the daytime than at night might result from more successful dodging of the net in the better illuminated upper layers of water. A greater

absolute number, however, could result only from downward migration in the daytime, providing the overall concentration were not greater. Such a downward migration might well occur as a result of negative phototropism. Comparison of the night and day hauls for stations 2452 and 2454 (Figure 37) reveals that there actually were more larvae at the deeper levels in the day hauls. Four larvae were taken in the day series at 59 meters, and one at 73 meters, while none were taken at either of these levels in the night series. The significance of these differences was tested by means of Bayes' theorem (using the formulation of Pearson, 1930, page lxx) considering a success the presence of a larva at the indicated depth, and assuming the same overall concentration for day and night (in other words assuming that the lesser numbers in the upper layers in the daytime were due to dodging of the net). The computation gave values of P of .06 at 59 meters and .50 at 73 meters. The latter, of course, does not indicate a significant difference between day and night hauls, but the former, closely approaching the conventional significance level of .05, gives some indication of an actually deeper distribution in the daytime.

In applying this result to the prediction of vertical distribution, a knowledge of the penetration of light at various times of day is necessary. Unfortunately, no empirical data for the area of our survey are available, and we are forced to argue by analogy with data collected elsewhere. G. L. Clarke (1934) has shown in his graphs that submarine irradiation in the Atlantic Ocean reaches nearly its maximum value shortly after sunrise, and is maintained there until shortly before sunset. Accordingly, extension of the regular hauls below 73 meters between sunrise and sunset would seem to be necessary in order to make sure of sampling all strata in which larvae were to be found.

SUMMARY AND CONCLUSIONS

Analysis of catches of pilchard eggs and larvae taken in serial hauls in 1939 and 1941 indicates the following relationships and effects:

1. A positive correlation of concentrations of eggs and larvae with temperature, at least within the range of 10° to 17° c.
2. An apparent dodging of the nets by the larger larvae, in the layers of water which are illuminated in the daytime.
3. A negative phototropism for the larger larvae.

Such relationships with physical conditions as have been indicated by the present study may, of course, hold true only for the particular set of oceanographic conditions prevailing at the times and places where the data were gathered. For instance, the relationship between the horizontal distribution of pilchard eggs and temperature as indi-

cated by our regular spawning surveys for 1941 was quite different from that found in 1940. However, the data for the present study were gathered in two different years (1939 and 1941) and over a considerable area, so that they may permit of some generalization.

It may have been noted that the concentrations of eggs and larvae are correlated with depth as well as temperature, since the latter two are themselves highly correlated. However, there are some instances where the deeper extension of warm water corresponds with a deeper distribution of eggs or larvae (cf. Figure. 35). Also, there is some logical basis for a correlation with temperature, which might act either as the cause of direct reaction of the larvae, or as an indicator of density differences affecting the distribution of eggs.

It is not intended to argue that temperature itself necessarily controls the vertical distribution of pilchard eggs and larvae. Their location may be determined by complex reactions of the spawners to a combination of physical conditions, including salinity, fodder food, density etc. To the extent that these would induce spawning in a homologous portion of the layer of water above the thermocline, temperature may be merely an indicator and not a cause. This, of course, does not detract from the usefulness of temperature as a guide to vertical distribution.

As a basis for predicting the proper depths to which to send nets on regular surveys, the regressions of egg and larvae concentrations on temperature indicate that hauls should be made deep enough to go below temperatures of 10.5° C. for eggs and 12.0° for larvae. Distribution of the large larvae during the daytime shows the need for fishing to a depth greater than 73 meters between sunrise and sunset.

PILCHARD EGGS AND LARVAE TAKEN IN VERTICAL SERIAL HAULS BY "E. W. SCRIPPS"

Data are given in the following order: Station number; date; time interval; latitude; longitude; type of net; depth interval for each haul; numbers of eggs; length and number of larvae for larvae of each .5 mm. interval of length. Time of day is given to the nearest hour with the hours numbered consecutively from 1 for 1 AM to 24 for midnight. Depths are in meters and lengths of larvae in millimeters.

Example: (Station) 1832; (Month) 4/ (Day) 10/ (Year) 41; (Start) 11h– (End) 15h: (North latitude) 33:13; (West longitude) 118:26; (Type of net) B; (Upper limit of stratum fished, depth) 0– (Lower limit of stratum fished, depth) 13: (Number of eggs taken) E 181; (Larvae) L: (Length) 2.5, (Number) 3; (Length) 3.0, (Number) 35; (length) 3.5, (Number) 4; (Limits) 17–26: (Number of eggs) E 103:

(Larvae) L: (Length) 2.5, (Number) 22; (Length) 3.0, (Number) 366; etc.

Net types are as follows: Type A is a closing net of one meter diameter mouth; made of No. 24XXX grit gauze, with the last half meter of No. 56XXX grit gauze; and with a detachable "cod end" of No. 56XXX grit gauze. Type B is a closing net of one-half meter diameter mouth; made of No. 40XXX grit gauze, with the last 65 cm of No. 56XXX grit gauze. Type C is a closing net of one meter diameter of mouth; made of cotton scrim with openings approximately 1 mm. square, with the last half meter of No. 56XXX grit gauze; and with detachable "cod end" of No. 56XXX grit gauze. Approximate sizes of openings of XXX grit gauzes are: No. 24, 1.10 mm., No. 40, .65 mm.; No. 56, .47 mm.

F8A; 4/19/39; 3h–9h; 32:48, 117:42; A; 0–3: E 1877; L: 4.0, 1; 4.5,1; 5.0,4; 5.5,2; 7.0,2; 8.0,1; 9.5,1; 6–8: E 2879; L: 4.0,1; 4.5,3; 5.0,7; 6.0,1; 6.5,2; 7.0,1; 7.5,1; 8.0,2; 9.0,2; 10.0,1; 10.5,1; 7–16: E 3878; L: 3.0,2; 3.5,1; 4.0,3; 4.5,8; 5.0,9; 5.5,2; 6.0,2; 17–25: E 1265; L: 5.0,1; 8.0,1; 30–34: E 56; L: 0; 44–46: E 5; L: 0; 45–60: E 0; L: 4.5,2; 108–115: E 6; L: 0.

F8B; 4/19/39; 15h–19h; 32:48, 117:44; A; 0–2: E 2892; L: 5.5,1; 6.5,1; 7.0,1; 15–17: E 1359; L: 3.5,1; 4.5,1; 29–35: E 88; L: 0; 53–58: E 8; L: 0; 68–72: E 0; L: 0; 164–183: E 4; L: 0.

F9A; 4/20/39; 2h–8h; 32:30, 117,32; A; 0–5: E 52; L: 3.5,6; 4.0,1; 4.5,2; 5.0,4; 5.5,6; 6.0,1; 6.5,3; 7.0,2; 12–14: E 194; L: 6.0,1; 28–32: E 54; L: 0; 45–57: E 1; L: 6.0,1; 56–59: E 1; L: 0; 109–*: E 7; L: 0.

F9B; 4/20/39; 9h–13h; 32:29, 117:39; A; 0–2: E 940: L: 5.5,2; 6.0,4; 6.5,5; 7.0,7; 8.0,3; 8.5,1; 9.0,1; 9.5,2; 10.0,1; 11.0,1; 14–*: E 4137; L: 3.5,1; 4.0,6; 5.0,1; 6.0,1; 57–66: E 6; L: 0; 126–149: E 4; L: 0.

1832; 4/10/41; 11h–15h; 33:13, 118:26; B; 0–13: E 181; L: 2.5,3; 3.0,35; 3.5,4; 17–26: E 103; L: 2.5,22; 3.0,366; 3.5,187; 4.0,2; 4.5,6; 5.5,1; 28–39: E 11; L: 2.5,1; 3.0,47; 3.5,31; 4.0,2; 4.5,6; 5.0,2; 41–55: E 0; L: 0; 53–66: E 0; L: 3.0,2; 3.5,2; 69–83; E 0; L: 2.5,1; 3.5,1; 5.0,1.

2046; 4/30/41; 17h–21h; 32:29, 119:26; B; 0–3: E 0; L: 3.0,3; 3.5,3; 4.0,1; 4.5,2; 5.0,33; 5.5,36; 6.0,9; 6.5,1; 7.0,1; 9.5,1; 6–12: E 0; L: 3.5,2; 4.0,2; 4.5,2; 5.0,31; 5.5,51; 6.0,55; 6.5,20; 7.0,3; 7.5,4; 8.0,2; 8.5,2; 9.0,1; 9.5,1; 10.0,2; 14.0,1; 15–25: E 0; L: 2.5,1; 3.0,1; 3.5,2; 4.0,1; 4.5,3; 5.0,27; 5.5,37; 6.0,74; 6.5,23; 7.0,9; 7.5,5; 8.0,5; 8.5,2; 9.0,2; 9.5,2; 10.5,1; 14–29: E 0; L: 3.0,2; 3.5,11; 4.0,11; 4.5,12; 5.0,38; 5.5,29; 6.0,15; 6.5,3; 28–43: E 0; L: 2.5,3; 3.0,19; 3.5,30; 4.0,16; 4.5,11; 5.0,25; 5.5,3; 6.0,6; 6.5,3; 7.5,1; 44–62: E 3; L: 2.5,1; 3.0,13; 3.5,11; 4.0,4; 4.5,1; 5.0,1; 65–92: E 18; L: 6.0,1.

* Only one stray angle reading.

2043; 5/2/41; 20h–24h; 32:50, 118:18; C; 0–4: E 90; L: 3.0,1; 5.0,20; 5.5,30; 6.0,32; 6.5,20; 7.0,38; 7.5,43; 8.0,53; 8.5,48; 9.0,33; 9.5,17; 10.0,19; 10.5,6; 11.0,14; 11.5,13; 12.0,7; 12.5,4; 13.0,5; 13.5,3; 14.0,1; 14.5,1; 15.0,2; 7–13: E 16; L: 4.0,1; 4.5,4; 5.0,21; 5.5,19; 6.0,12; 6.5,6; 7.0,10; 7.5,9; 8.0,14; 8.5,15; 9.0,23; 9.5,13; 10.0,26; 10.5,12; 11.0,7; 11.5,11; 12.0,15; 12.5,9; 13.0,7; 13.5,6; 14.0,3; 14.5,3; 15.0,1; 16.0,1; 17–26: E 1; L: 4.5,1; 5.0,2; 5.5,1; 6.0,1; 7.0,1; 9.5,1; 11.5,1; 12.5,1; 13.5,1; 25–33: E 1; L: 4.5,1; 11.5,1; 12.0,1; 14.0,2; 14.5,1; 15.5,1; 30–53: E 0; L: 11.5,1; 52–73: E 0; L: 0; 68–98: E 0; L: 0; 97–142: E 0; L: 0.

2452N; 6/17–18/41; 22h–3h; 32:23, 117:52; C; 0–3; E 1; L: 11.0,1; 12.0,5; 12.5,4; 13.0,3; 13.5,3; 14.0,2; 14.5,1; 15.5,1; 18.5,1; 7–12: E 0; L: 8.5,1; 13.5,1; 14.0,1; 15–24: E 0; L: 9.0,2; 11.0,2; 11.5,1; 12.0,1; 12.5,2; 13.0,1; 13.5,1; 15.0,2; 22–35: E 0; L: 13.0,1; 36–52: E 0; L: 10.5,1; 11.5,1; 14.5,1; 48–84: E 0; L: 0; 57–101: E 0; L: 0; 90–146: E 0; L: 0.

2452D; 6/18/41; 9h–14h; 32:17, 117:52; C; 0–3: E 0; L: 0; 6–12: E 0; L: 0; 14–21: E 0; L: 0; 25–36: E 0; L: 11.5,1; 38–43: E 0; L: 11.5,1; 12.5,1; 52–67: E 0; L: 17.5,1; 18.0,1; 68–81: E 0; L: 18.0,1; 96–152: E 0; L: 0; 125–156: E 0; L: 0.

2454N; 6/18–19/41; 21h–2h; 32:12, 118:38; C; 0–4: E 8; L: 4.5,1; 5.0,2; 6.0,1; 6.5,2; 7.0,1; 9.0,1; 9.5,1; 10.0,1; 10.5,3; 12.5,1; 7–13: E 15; L: 4.5,1; 5.0,2; 6.0,1; 6.5,2; 8.5,1; 9.0,2; 10.5,1; 11.0,1; 12.0,1; 12.5,1; 18–25: E 1; L: 5.0,1; 7.5,1; 8.0,1; 9.0,3; 9.5,4; 10.0,1; 11.0,2; 12.0,2; 12.5,1; 13.0,1; 13.5,1; 14.0,2; 14.5,2; 15.0,1; 24–32: E 0; L: 4.5,5; 5.0,2; 5.5,1; 7.0,2; 8.5,1; 9.0,2; 9.5,3; 11.0,5; 11.5,3; 12.0,3; 12.5,3; 13.0,4; 17.5,1; 38–52: E 0; L: 10.0,1; 11.0,2; 12.5,1; 52–69: E 0; L: 0; 66–108: E 0; L: 0; 90–149: E 0; L: 0; 122–208: E 0; L: 0.

2454D; 6/19/41; 8h–13h; 32:10, 118:39; C; 0–3: E 3; L: 6.0,1; 10.0,2; 12.0,1; 12.5,1; 7–14: E 1; L: 0; 17–25: E 0; L: 4.5,1; 6.0,3; 6.5,1; 10.0,1; 23–36: E 0; L: 4.0,2; 4.5,4; 5.5,2; 13.0,1; 36–47: E 0; L: 5.0,1; 12.0,1; 52–69: E 0; L: 21.0,1; 26.0,1; 70–85: E 0; L: 0; 99–121: E 0; L: 0; 120–198: E 0; L: 0.

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THE RATIO OF CARBON DIOXIDE CONSUMPTION TO OXYGEN EVOLUTION IN SEA WATER IN THE LIGHT[1]

By

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The production of organic matter in sea water by the phytoplankton community has frequently been computed (Nielsen, 1937; Riley 1941) by measuring the oxygen evolution in bottled sea water samples and calculating the organic matter on the assumption that the phytoplankton organisms produced glucose by photosynthesis according to the equation:

$$6CO_2 + 6H_2O \rightleftharpoons C_6H_{12}O_6 + 6O_2.$$

According to this assumption CO_2/O_2, that is, the ratio of carbon dioxide consumed to oxygen evolved in the light, is 1.0. Thompson and Gilson (1937, p. 31) have pointed out that as phytoplankton tissue contains nitrogen and carbon atoms in the ratio of one to seven, one fifth of the oxygen produced must originate from nitrate and the ratio CO_2/O_2 should be 0.8. On the other hand, Redfield (1934, p. 182) found that in the oxidation of organic matter in deep water the ratio of the carbon dioxide produced to the oxygen consumed was 1.17, but he considered the accuracy of the determination low.

In the experiments described below, the consumption of carbon dioxide and the formation of oxygen were both measured on the same samples of sea water exposed to natural illumination for a day.

Sea water was collected directly in a twelve liter Pyrex bottle, and two 4600 ml bottles with tight stoppers were completely filled from this reservoir through a siphon. Usually two collections were made and two pairs of bottles filled at a time. One bottle of each pair was wrapped in several layers of khaki duck and the four bottles were hung from the end of the pier in a frame at a depth below the surface varying from one to three meters according to the stage of the tide, and almost five meters from the bottom. After twenty-four hours the bottles were removed to a dark constant temperature room kept usually at 23° ± 0.5° C. (somewhat warmer than the sea), and allowed eighteen hours to come to temperature equilibrium.

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 198.

The concentration of carbon dioxide was calculated from a measurement of the pH using the data of Moberg, Greenberg, Revelle, and Allen (1934, p. 269). The pH measurements were made in a vessel of about 250 ml capacity arranged so that it could be filled from the experimental bottle through a siphon and emptied through another siphon. In this way the vessel could be repeatedly rinsed and filled with a minimum contact of the water with air. The pH was measured electrometrically by means of a glass electrode with a silver-silver chloride reference electrode. The latter was prepared by electrolytically plating a silver wire with silver chloride in a sodium chloride solution.

The silver-silver chloride electrode was used in preference to a calomel electrode because of the uncertain junction potentials often characteristic of the latter. More comprehensive data on the use of these electrodes in sea water is to be published at a later date. The measuring circuit consisted of a Leeds and Northrup type K potentiometer in conjunction with a Beckman No. 1100 electrometer. The precision of measurement when corrections were made for small temperature differences between the pairs of samples was approximately $\pm$ 0.1 mv or $\pm$ 0.0017 pH for each pair. In two cases the differences between light and dark bottles were so great that measurements were made with a Beckman pH meter.

After the completion of the electrometric measurement, samples were siphoned into 150 ml bottles and the oxygen concentration was measured by the Winkler method. The standard deviations of differences between the means of triplicate titrations varied from 0.02 to 0.07 milliliters of oxygen per liter.

In the table are shown the data for nine sets of observations. The mean of the nine resulting figures for the CO_2/O_2 ratio is 0.95 which, in view of the relatively large errors involved in the measurement of these small quantities, cannot be considered as significantly different from 1.0. With these data on the activities of raw sea water in the light can be compared Barker's (1935) data showing that the photosynthetic quotient of a pure culture of *Nitzschia closterium* lay between 0.93 and 0.96. The value found by Barker for this diatom appears from our measurements to be characteristic of the whole phytoplankton community. Both Barker's data and ours lead to the conclusion that fats constitute only a small part of the products of the photosynthesis of marine phytoplankton. In the practical problem of calculating the production of organic matter in the sea from observations on oxygen evolution the uncertainties are so much greater than this 5% difference that the ratio 1.0 can be used without hesitation.

MEASUREMENTS OF CARBON DIOXIDE CONSUMED AND OXYGEN EVOLVED BY RAW SEA WATER

	Temperature in °C	*Potential in millivolts*	*Difference in millivolts*	*Difference in alkalinity*		*Difference in CO_2 concentration ml./l.*	*Concentration of O_2 ml./l.*	*Difference ml./l.*	*Ratio of CO_2 consumed to O_2 evolved*
				pH	*ΔpH*				
July 20 light		−35.11					5.44		
" " dark	22.9	−33.61	1.50		0.0255	0.34	5.11	0.33	1.03
" " light		−35.00					5.40		
" " dark	22.8	−33.98	1.02		0.0174	0.23	5.11	0.29	0.79
" 25 light		−36.85					5.00		
" " dark	22.8	−35.60	1.25		0.0213	0.28	4.68	0.32	0.88
" " light		−36.11					5.03		
" " dark	22.8	−35.23	0.88		0.015	0.20	4.81	0.22	0.91
" 28 light		−35.85					5.30		
" " dark	22.8	−35.50	0.35		0.0060	0.08	5.17	0.13	0.6
Aug. 20 light		−30.94					5.02		
" " dark	19.5	−30.40	0.54		0.0093	0.12	4.88	0.14	0.88
" " light		−31.22					5.03		
" " dark	19.5	−30.75	0.59		0.0101	0.13	4.91	0.12	1.1
Sept. 29 light				8.78			8.04		
" " dark	20.8			8.56	0.22	4.6	4.27	3.77	1.2
" " light				8.73			9.00		
" " dark	20.8			8.58	0.15	3.2	6.25	2.75	1.2

The correction suggested by Thompson and Gilson is probably not practically significant with the techniques of measuring organic production in their present condition. However, it is worth considering in the present connection.

In Barker's experiments using artificially dense populations of diatoms the amount of nitrate present initially was so small that the total nitrate reduction was negligible compared with the total carbon dioxide reduction during the period of the observations. Our observations on raw sea water were made late in the season when regeneration of nitrate was presumably occurring nearly or quite as fast as nitrate reduction. If this is true, careful observations should show an annual cycle in the value of CO_2/O_2.

The two nearly identical values found on September 29 merit special consideration. They were obtained during a period of red water caused by a bloom of a *Gonyaulax* sp. when the carbon dioxide consumption and oxygen evolution were about ten times as large as during the other observations. The uncertainty of the values for CO_2/O_2 is not more than $\pm$ 5%. Another feature of the conditions at this time is that the total photosynthesis (difference in final oxygen content between the light bottle and the dark bottle) was only about 10% higher than the respiration (difference between the initial and final oxygen contents of the dark bottle) while during the other observations it was two to four times the respiration. Under these conditions such high values for the ratio CO_2/O_2 could occur if (1) the product of photosynthesis were a carbohydrate, and, (2) the product of respiration were in part a keto-acid, hydroxy-acid or other highly oxygenated substance. If the uncertainty of the values of the ratio CO_2/O_2 in these sets of observations were great, the hypothesis would not be worth formulating, but under the circumstances there is a strong presumption that a fermentation of the kind described was actually occurring on a measurable scale.

CONCLUSIONS

1. The ratio of carbon dioxide consumed to oxygen evolved in the photosynthetic processes of the phytoplankton of the sea is not significantly different from 1.0 during the season of high phytoplankton productivity.

2. Fermentative processes resulting in incomplete oxidation products of carbohydrates may occur on a measurable scale when the organic content of sea water is high.

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THE EFFECT OF COPPER UPON THE DEVELOPMENT OF BACTERIA IN SEA WATER AND THE ISOLATION OF SPECIFIC BACTERIA[1]

By

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INTRODUCTORY AND HISTORICAL

Copper is used extensively in the eradication of various types of disease-producing microorganisms, especially fungi, as well as other organisms which are a nuisance to water supplies, such as algae and certain animal forms. It is also known that traces of copper are essential for the growth of many of the lower forms of life. The extensive literature on the relation of copper to microbial development is largely limited to the above two phenomena. Comparatively little is known, however, of the effect of copper upon a mixed microbiological population consisting of many organisms with different metabolic processes. It is known, for example, that the growth of plants in certain soils, as peats, will respond markedly to the application of small amounts of copper (5). The development of fungi in copper-free media has been suggested as a means of determining the concentration of copper in a given soil; this method is based upon the response of *A. niger* to the presence of small amounts of available copper (3).

Certain microorganisms are known to be capable of tolerating fairly large amounts of copper. However, the organisms investigated so far are found largely among the fungi (1, 6, 7, 8). What role these organisms play in the transformation of copper in nature or even in artificial substrates is still a matter of speculation; high copper concentrations retard the growth of the organisms during the early stages of development. The presence of excessive concentrations of copper brings about the elimination of most microorganisms in a natural substrate with the exception of certain fungi capable of withstanding these high copper concentrations (6). The mycelium of some of the

[1] Contribution No. 325 of the Woods Hole Oceanographic Institution and Journal series paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology.

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copper-resisting fungi was found (1) to contain considerable copper, which is in a fixed state and cannot be readily removed by acids. The absorption of copper during the early stages of growth was found (4) to be followed by the elimination of the copper into the medium in the older cultures.

The relationship of copper to bacteria has been chiefly limited to its disinfectant action, different bacteria varying greatly in their resistance to copper. Some of the bacteria were found (2) to take up enough copper to color them, first green, then brown.

A search for information concerning the relationships of marine bacteria to copper in sea water suggested the desirability of undertaking a study of the effect of copper upon marine bacteria as a whole and of the presence in the sea of bacteria capable of withstanding copper in concentrations greater than mere traces. Particular attention was paid to the influence of copper on the development of marine bacteria in sea water and in culture media. The problems of adjustment of bacteria to varying copper concentrations in sea water and the effect of these bacteria upon the copper have been studied.

EXPERIMENTAL

The effect of copper on marine bacteria. The medium selected for the study of the effect of copper upon marine bacteria was one extensively used in this laboratory for the study of marine bacteria and supplemented with varying amounts of $CuSO_4.5H_2O$. This medium has the following composition:

Agar	15 gm
Glucose	1 gm
Peptone	1 gm
K_2HPO_4	0.05 gm
Sea water	1000 ml

The medium was divided into several portions which received additions of $CuSO_4$ ranging from 0 to 40 mg per liter. These media were tubed, plugged, and sterilized and used for plating out fresh sea water. The numbers of bacteria found in the water taken near the dock of the Oceanographic Institution at Woods Hole showed no appreciable differences in the media receiving the above amounts of copper. Apparently these concentrations were not great enough to have an inhibiting effect upon the growth of marine bacteria. Even in this preliminary experiment, however, the appearance of several red-brown pigmented colonies on the plates containing the largest amount of $CuSO_4$ was observed.

This experiment was repeated using greater concentrations of copper in the medium, as follows:

Medium No.	$CuSO_4.5H_2O$ *mg/L*
1	0
6	50
7	100
8	200

Fresh sea water was plated out in dilutions of 1 and 1 : 10 using the above media. In addition, small amounts of fresh films from plates submerged in the water for several weeks were also plated out in dilutions of 1 : 1,000 and 1 : 10,000. The plates were incubated for two and four days at room temperature (25° C.), and the total colonies developing on the plate were determined. The red-brown colonies, which had a typical appearance of metallic copper, were designated as "copper colonies." The results obtained may be summarized as follows:

NUMBERS OF BACTERIA IN 1 ML. FRESH SEA WATER

Medium No.	*Colonies per plate, dilution* 1 : 10	
	Total colonies	*"Copper" colonies per plate*
1	200	0
6	160	10
7	3	0
8	0	0

NUMBERS OF BACTERIA IN A SMALL AMOUNT OF FILM FROM SUBMERGED SURFACE

Medium No.	*Colonies per plate,* 1 : 1,000 *dilution*	
	Total colonies	*"Copper" colonies*
1	>500	0
6	>500	a few
7	500	many
8	90	0

On continued incubation of the plates, the red color of the "copper" colonies on the media containing $CuSO_4$ disappeared, the colonies losing their red pigment and becoming white again.

There was a slight increase in acidity of the medium with an increase in copper concentration; however, even the highest concentration of copper used in this experiment did not make the medium very acid, changing the pH to 6.9. It is believed that these copper concentrations would not have had a great enough effect upon the growth of the bacteria merely through the pH change.

It was apparent from the results of these experiments that the presence of $CuSO_4$ in the medium was responsible for the pigmented colonies, and the presence of 200 mg of $CuSO_4.5H_2O$ per liter of

medium was too great a concentration for the development of the total bacterial population in the water, as well as of the specific "copper" present. The addition of 100 mg $CuSO_4.5H_2O$ per liter of medium was found to be the optimum for the development of the specific "copper" colonies on the plate. This concentration was used in the subsequent experiments for determining the abundance of the "copper" organisms in sea water. The medium containing this amount of copper will henceforth be referred to as medium 7.

In order to determine whether such "copper" bacteria are also present in soil and in lake water, samples of ordinary soil and pond water were plated out, using nutrient agar media enriched with 100 mg of $CuSO_4.5H_2O$ per liter. The materials were plated out using different dilutions. The numbers of bacteria obtained on this medium were 1,600 per ml of pond water and 60,000 per gm of soil, after 2 days incubation. No typical "copper" colonies appeared on any of the plates. Whether these bacteria are totally absent in soil and in fresh water or whether the salts present in the media used for plating out the sea water had a specific effect upon the development of such bacteria, still remains to be determined.

The effect of additions of various forms of copper to sea water upon the general bacterial population of the water as a whole and upon the development of specific "copper" bacteria was now studied.

The influence of $CuSO_4$ *upon the bacteria and upon oxygen consumption in sea water.* The total numbers of bacteria in the water were measured by the plating method, using the copper-free medium 1; the number of "copper" bacteria were determined by the use of medium No. 7. Oxygen consumption in the water, as influenced by the addition of varying amounts of copper, was also measured by the common Winkler method.

Several preliminary experiments were carried out for the purpose of determining the type of container to be used for incubating the water, the method for treating the water, and the length of the incubation period (Table I).

Only the results of one typical preliminary experiment are reported here. Measured quantities of fresh sea water received varying amounts of $CuSO_4.5H_2O$. They were immediately distributed in oxygen bottles (230 ml portions), which were incubated under water at room temperature (20°–25° C). After different periods of incubation, the bottles were removed and contents used for plating and oxygen determination. One ml portions were removed with sterile pipettes, disturbing the water in the bottles as little as possible. The

TABLE I—Influence of Copper upon the Development of Bacteria and Consumption of Oxygen in Fresh Sea Water Kept in Laboratory

Incubation of water, days	*Copper added as $CuSO_4.5H_2O$ mg/l.*	*Oxygen consumed ml/l.*	*Total numbers of bacteria per 1 ml water*	*"Copper" bacteria per 1 ml water*
0	0	0	2.000	.45
1	0	1.204	3.160.000	232.000
1	1	0.777	1.660.000	720.000
1	10	0.018	38.000	6.500
1	50	0.021	73.500	5.000
1	200	0.070	105.000	17.000
2	0	1.708	2.400.000	35.000
2	1	1.544	4.400.000	166.000
2	10	0.679	1.050.000	163.000
2	50	0.665	1.550.000	115.000
2	200	0	24.000	1.500
4	0	2.216	220.000	1.000
4	1	2.590	3.440.000	21.500
4	10	0.952	3.760.000	42.500
4	50	0.892	6.540.000	290.000
4	200	0.574	703.000	682.000
7	0	2.976	200.000	2.000
7	1	4.480	22.000	1.500
7	10	0.952	2.510.000	1.050.000
7	50	0.795	650.000	360.000
7	200	0.795	810.000	31.500

reagents for the oxygen determination were then added to the residual water in the bottles. The bacterial plates were incubated at room temperature for 4 days and counted at the end of the 2d and 4th days. The 4 day counts are reported unless otherwise indicated.

Although the results of this experiment are somewhat erratic, nevertheless they permit certain interesting conclusions. The addition of copper to the water had at first a depressing effect upon bacterial development and oxygen consumption. This effect was soon overcome; the smaller the amount of copper added the shorter was the period of "depression" or "adaptation." Subsequently copper had a stimulating effect, depending on the amounts of copper added; the less the concentration of copper the sooner the stimulating effect was observed. At the highest concentration of copper (200 mg $CuSO_4.5H_2O$), the period of depression was longest and was not completely overcome even after 7 days incubation, the longest period used in this experiment.

In order to overcome the variability in the results which definitely appeared to be due to experimental errors, this experiment was repeated, using a larger number of replicates and extending the incubation period for a somewhat longer time. Fresh sea water, taken some 100 feet away from the pier of the Oceanographic Insti-

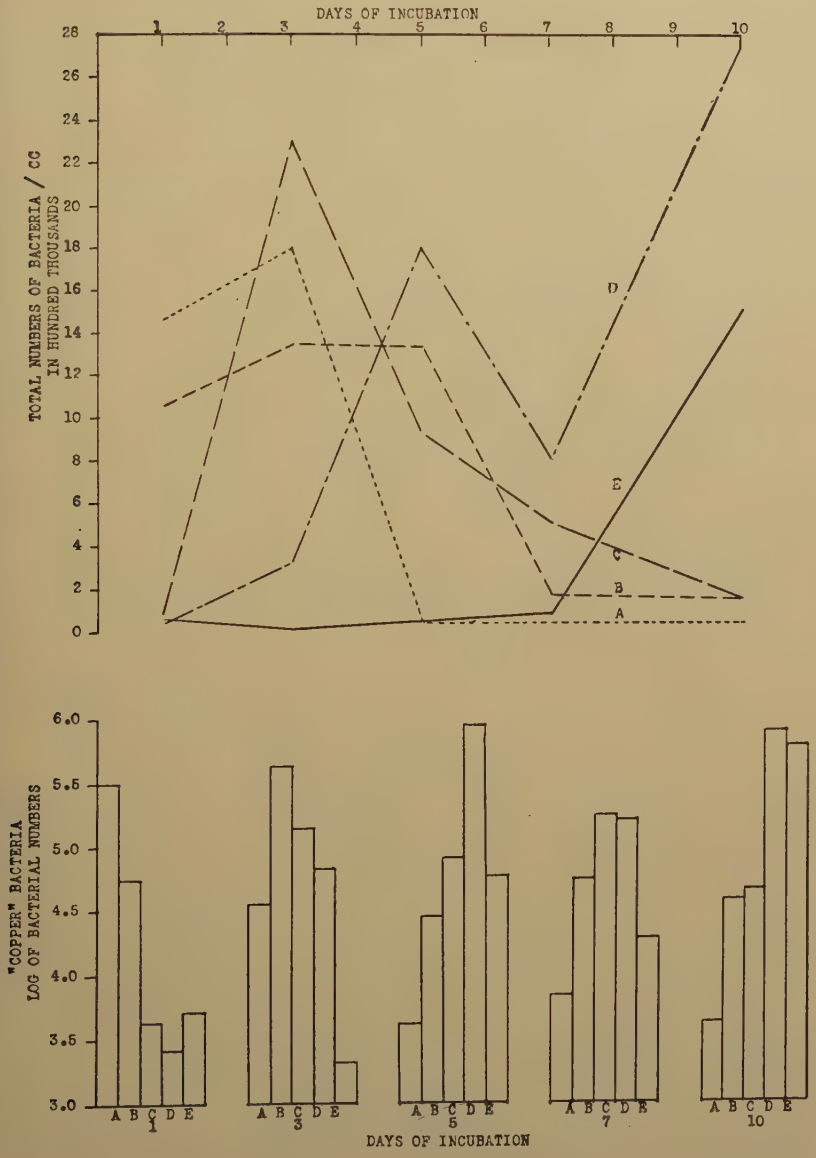

Figure 38. Influence of copper sulfate upon the total bacterial population of sea water and upon the development of "copper" bacteria. $CuSO_4.5H_2O$/L Sea water: (A.) none; (B.) 1 mg; (C.) 10 mg; (D.) 100 mg; (E.) 500 mg.

tution, was placed in fifty oxygen bottles, each containing about 150 ml water. These bottles were divided into 5 groups of 10 each. Group A received no copper, B—1 mg $CuSO_4.5H_2O$ per liter of fresh water, C—10 mg, D—100 mg, and E—500 mg $CuSO_4.5H_2O$. The

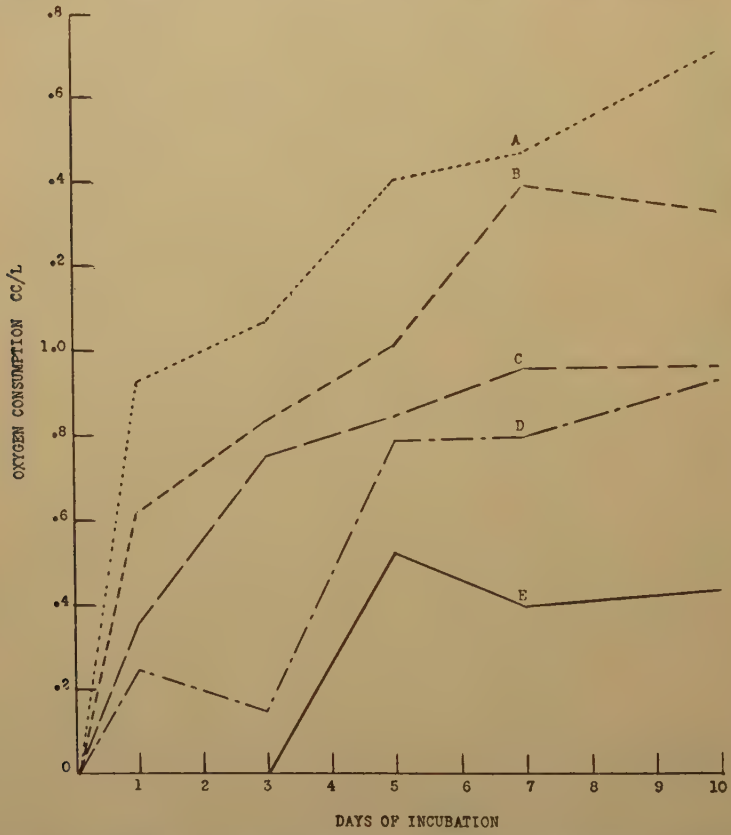

Figure 39. Influence of copper concentration upon the consumption of oxygen in sea water. $CuSO_4.5H_2O$/L: (A.) none; (B.) 1 mg; (C.) 10 mg. (D.) 100 mg; (E.) 500 mg.

bottles were incubated under water. Two bottles from each lot were removed for analysis after 1, 3, 5, 7, and 10 days.

The original fresh sea water contained 670 bacteria and 90 "copper" bacteria per 1 ml of water. The oxygen content of the water was 5.09 cc/liter. Figures 38 and 39 present graphically the results of this experiment. Here again, the addition of copper to the water had a delaying effect upon bacterial development, which was later overcome;

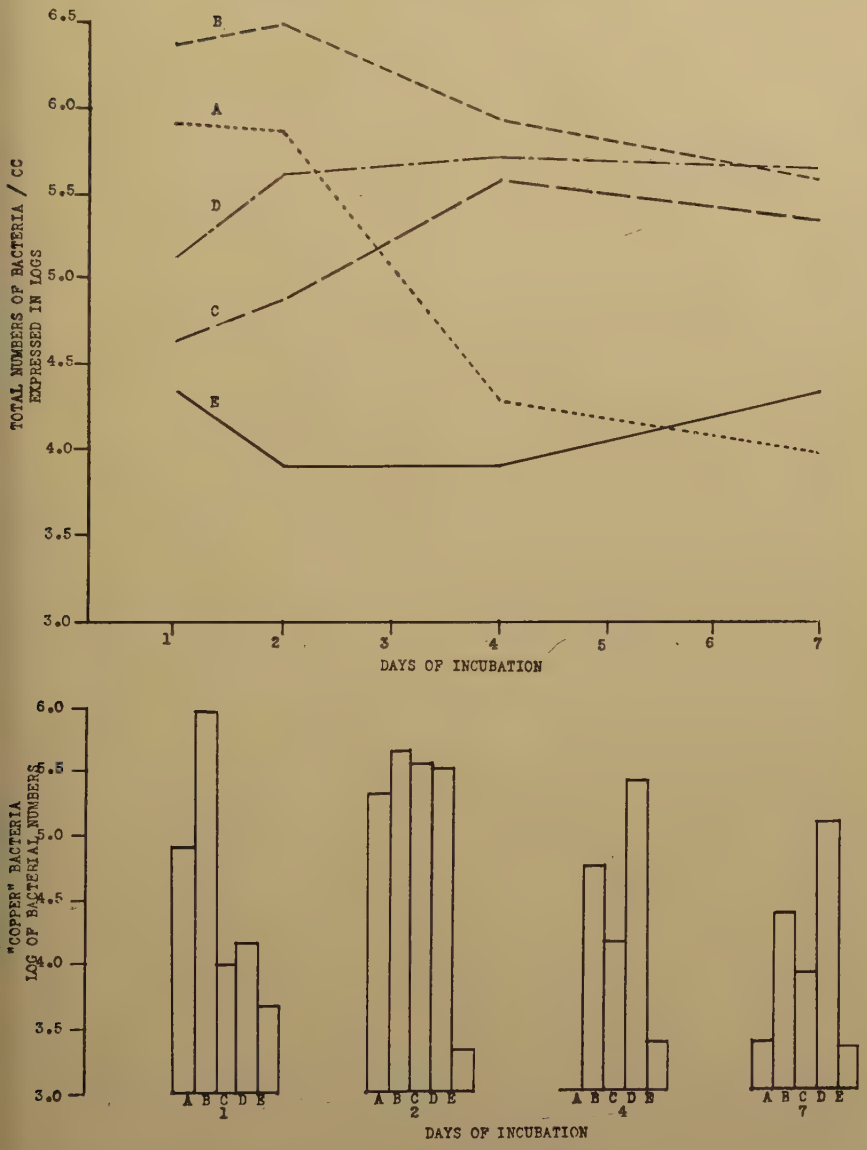

Figure 40. Influence of copper chloride upon the total bacterial population of sea water and upon the development of "copper" bacteria. $CuCl_2$/L; (A.) none; (B.) 1 mg; (C.) 10 mg; (D.) 50 mg; (E.) 100 mg.

the larger the amount of copper added, the greater was the length of the depression period. No explanation can be offered for the fact that curve B, for the water receiving the smallest amount of copper, did not show the stimulating effect obtained in the previous experiment; this may possibly be due to the omission of the two day incubation period, when such a rise might have been observed. The effect upon the consumption of oxygen in the water was also not stimulating but depressive, this effect increasing with an increase in the amount of copper added.

The influence of $CuCl_2$ *upon bacterial development and upon oxygen consumption in sea water.* In order to avoid the secondary effect of

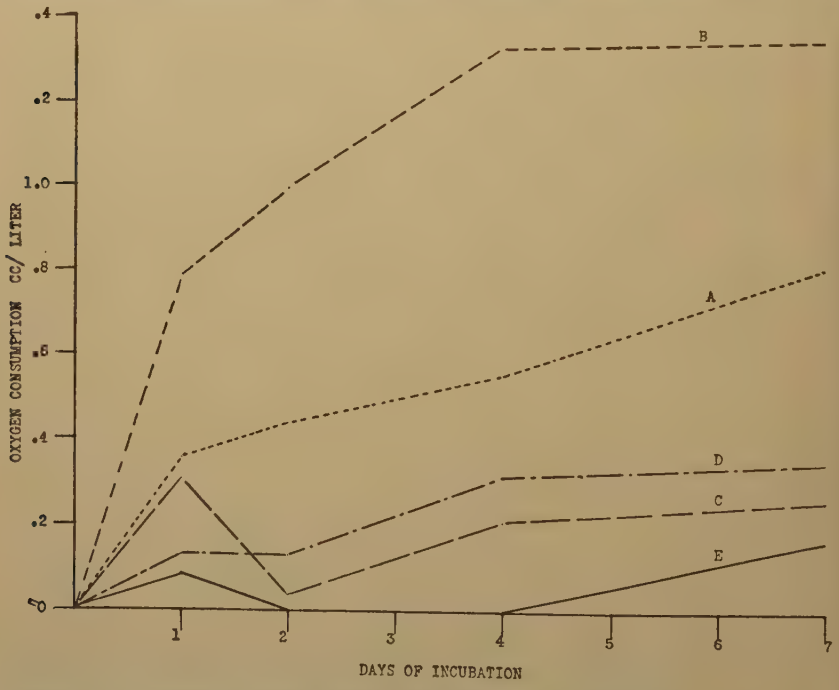

Figure 41. Influence of copper chloride upon the oxygen consumption by bacteria in sea water. $CuCl_2$**/L: (A.) none; (B.) 1 mg; (C.) 10 mg; (D.) 50 mg; (E.) 100 mg.**

the sulphate ion upon bacterial development, copper chloride was now employed. This experiment was carried out in a manner similar to the previous one. Five series of bottles were used. These received fresh sea water containing the following amounts of $CuCl_2.2H_2O$ per liter of water.

Series	$CuCl_2.2H_2O$ mg/L
A	0
B	1
C	10
D	50
E	200

The bottles were incubated as in the previous experiments and the bacterial numbers and oxygen consumption were determined. The bacteria were plated out on media 1 and 7, in dilutions of 1 : 1,000 and 1 : 10,000. The fresh sea water, plated out at the beginning of the experiment, gave a total of 16,960 bacteria per 1 cc of water using medium 1 and 1,840 "copper" bacteria on medium 7.

The results of this experiment (Figures 40 and 41) are very similar to those obtained with $CuSO_4$. Copper in the form of $CuCl_2$ had, in small quantities, a stimulating effect upon the growth rate of marine bacteria and especially upon oxygen consumption in the water. One mg. of $CuCl_2.2H_2O$ per liter of sea water gave a marked increase in the total numbers of bacteria as well as in the numbers of "copper" bacteria. The addition of 10 mg or more of the copper salt per liter of water showed, however, marked inhibiting effects, which tended to be overcome on further incubation. The largest amount of copper salt added had the greatest depressive effect; the period of depression was practically ended after 7 days' incubation of the water in the laboratory.

The influence of Cu_2O *on bacterial development and oxygen consumption in sea water.* A third form of copper, Cu_2O, was now used for determining the effect upon bacterial activities in sea water. This experiment was carried out in a manner similar to the previous ones. An incubation period of 14 days was used.

The water was treated as follows:

Series	Cu_2O mg/L
A	0
B	1
C	5
D	20
E	100

The fresh sea water plated out at the beginning of the experiment gave 238 bacteria on medium 1 and 10 "copper" bacteria on medium 7 per 1 ml of water. The oxygen content of fresh water was 4.99 cc/liter. This is similar to that found in the other experiments; however, when the water was tested immediately after the Cu_2O was

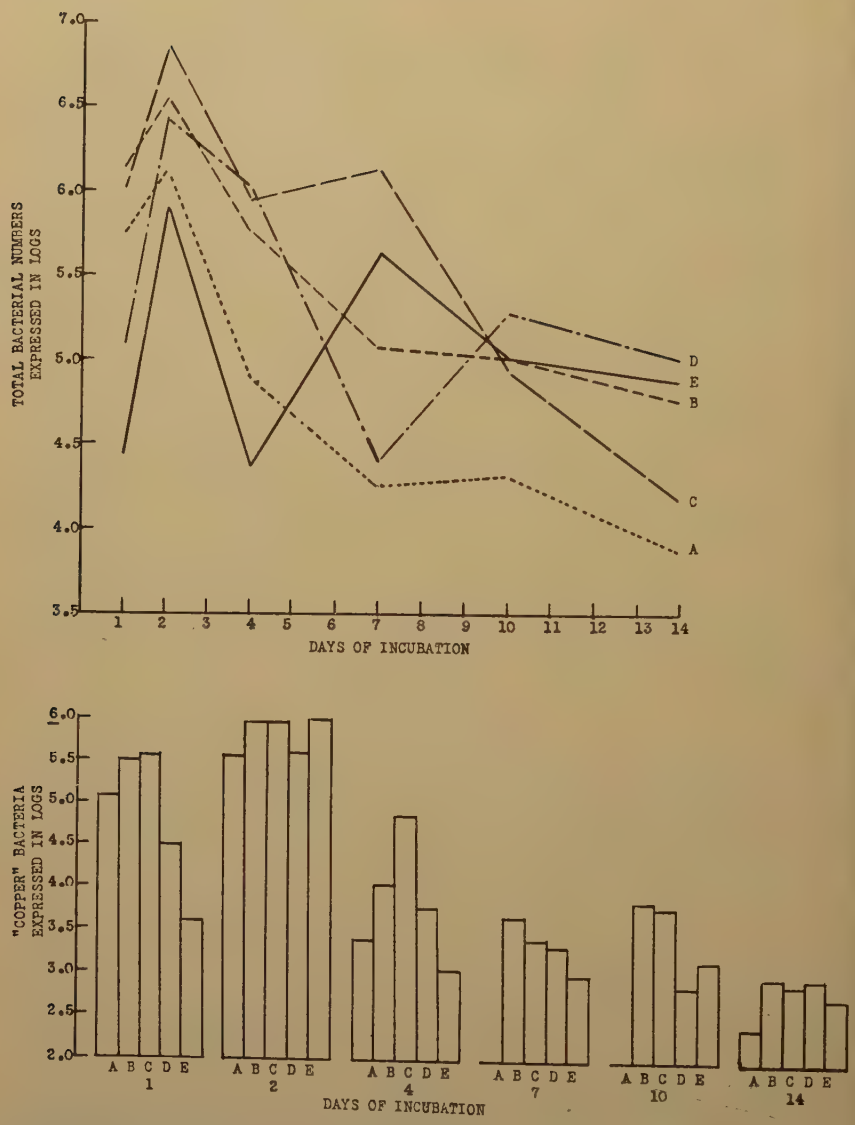

Figure 42. Influence of copper oxide upon the total bacterial population in sea water and the development of "copper" bacteria. Cu_2O/L sea water: (A.) none; (B.) 1 mg· (C.) 5 mg; (D.) 20 mg; (E.) 100 mg.

introduced, there was an immediate marked consumption of the oxygen. This was due to the oxidation of the cuprous oxide to cupric oxide. This reaction consumed approximately 1.5 cc of oxygen per 1 liter of sea water in the E series, to which the highest amount of Cu_2O was added.

This experiment was repeated in order to determine the amount of oxygen taken up chemically and biologically by the addition of Cu_2O to the fresh water. Two lots of bottles were filled with fresh sea water, one lot receiving no copper and the other receiving 100 mg of Cu_2O per liter of water. These bottles were incubated for short period of time and analyzed for oxygen. The results reported in Table II definitely point to the fact that the chemical consumption of the oxygen is immediate and does not increase on further incubation.

The results of the effect of Cu_2O on bacterial development presented in Figure 42 confirm fully the effects of the other two forms of copper, namely, at first a depression followed by a stimulating effect. The greater the amount of copper added, the longer was the depressive action. There were, however, two exceptions; first, no depressive action was observed where the two smallest amounts of copper were added as Cu_2O; second, the depressive effect of the largest amount of copper was attained with the 7 day incubation period.

TABLE II—Chemical and Biological Oxygen Consumption in Water Treated with Cu_2O

Incubation of water, hours	*Water enriched with* Cu_2O	*Unenriched water*	*Biological Consumption*	*Chemical Consumption*
Start	3.41	5.00	0	1.59
2	3.32	4.98	0.02	1.66
4	3.21	4.93	0.07	1.72
6	3.16	4.93	0.07	1.77
8	3.16	4.88	0.12	1.72
10	3.16	4.73	0.27	1.57
24	3.16	4.34	0.66	1.18 (?)
72	2.28	3.82	1.18	1.54

100 mg Cu_2O per liter of water. Data expressed in cc of oxygen per liter of water

"Copper" bacteria in surface films and copper concentration by "copper" bacteria. A series of films removed from various surfaces which had been submerged near or at the dock of the Oceanographic Institution were examined for the total bacterial content and the percentage of "copper" bacteria. Uniform pieces of film were removed, by means of sterile swabs, suspended in sterile sea water, diluted and plated. The results (Table III) showed no particular uniformity between the total numbers and the numbers of "copper" bacteria. This may have been due to the fact that the films were of

different ages (42–170 days old) and varied considerably in thickness; the amounts of film removed from the different surfaces were also not absolutely uniform. The "copper" nature of some of the bacteria was not always recognized, since the pigmentation of the colonies and the disappearance of the pigment did not take place uniformly.

The results of these experiments point definitely to the fact that copper, in various forms, has some very definite relations to the development of bacteria in sea water. First, there is an apparent adaptation of the bacteria to the copper, whereby the latter may even exert a stimulating effect upon the development and activities of the marine bacteria, within definite limits of concentration and time. Second, there is a marked development, among the marine bacteria, of a specific population, which has been designated as "copper" bacteria; these are present in the water and are capable of developing there in very large numbers.

In order to demonstrate whether the copper is being concentrated in the bacterial pellicle, an attempt was made to produce such pellicles artificially. Sea water was enriched with the nutrients of medium 7 and placed in shallow layers in flasks. Some of these were left in an unsterile condition and others were sterilized and inoculated with some of the pure cultures of the "copper" bacteria. Typically red pellicles were produced in some of the flasks. These pellicles were carefully removed and analyzed for copper. It was found that about 8 per cent of the copper added to the medium was concentrated in the pellicles. Only a few analyses have been made so far, however, and further work is required in order to establish this point. Because of the great variability in the pigmentation of the pellicle and the limited analyses made so far, these results should be taken as merely suggestive. The oxygen tension of the medium appears to play an important role in this connection.

TABLE III—Numbers of Total Bacteria, Developing on Plate, and of "Copper" Bacteria in Comparable Units of Bacterial Films from Different Surfaces

	Medium 1 (no copper)	Medium 7 (copper present)	
No. of submerged plate	*Total numbers of bacteria*[1]	*Total numbers of bacteria*	*"Copper" bacteria*
0	428.000	57.000	8.000
10	1.295.000	361.500	28.500
14	1.680.000	124.500	20.500
20	1.095.000	102.500	7.000
25	1.150.000	114.500	12.000
28	930.000	103.500	21.500
33	400.000	107.000	4.000

[1] Per unit of surface removed by sterile swab.

Nature of the "copper" bacteria. A detailed study of the nature and physiology of the "copper" bacteria will be presented at a later date. It is sufficient to present here only some of their general characteristics in order to emphasize their specific nature.

The development of the "copper" colonies on the agar plate containing a copper salt was rather characteristic. The surface colonies behaved in this respect quite differently from the sub-surface colonies. The former produced round reddish to red-brown and even dark-red colonies; the pigment of the colonies gradually disappeared, either beginning from the center or from the periphery of the colony, until, after 5–6 days, the whole colony lost its red pigment. The sub-surface colonies kept the pigment for a longer period of time. This phenomenon did not appear to be correlated with sunlight. Whether the copper is at first assimilated or absorbed by the cell, then oxidized to give the pigmented form, and finally reduced or excreted remains to be determined. It was observed, however, that when a quantity of bacterial cell material was collected, dried and analyzed, it rapidly lost its red color and became greenish.

In order to study further the physiological characteristics, these bacteria were isolated in pure culture and cultivated on a special copper-containing medium. This medium was comparable to soft agar, thus enabling the organism to grow under partial anaerobic conditions. An extract of *Chondrus crispus* (Carragar) was used as the gel-forming agent. This medium, designated as 6b, had the following composition:

Peptone	1 gram
Glucose	1 gram
K_2HPO_4	0.05 gram
$CuSO_4.5H_2O$	0.1 gram
Carragar	6 grams
Sea water	1,000 ml.

This medium was placed in test tubes and sterilized. When the medium was allowed to set, it separated into two distinct layers; the lower layer was of a gel nature, whereas the top layer was in the form of a supernatant fluid. This enabled the bacteria to grow on the surface of the liquid; when a pellicle of sufficient thickness was produced, it dropped upon the lower or gel layer. The pellicle thus accumulated, without rapidly losing its red color, until several layers of film were formed, one on top of the other.

Colonies were picked from the various plates containing media 1 and 7 and inoculated upon this special medium. Both white and red colonies were picked from the copper-containing medium 7 and white colonies alone were picked from the copper-free medium 1.

These pure cultures were incubated in the dark at room temperature. The red color of the pellicle served as evidence of the "copper" character of the bacteria. Some cultures produced this coloration faster than others. Those colonies which were red on medium 7 gave red growth on medium 6b and those colonies that were white on medium 7, remained white on the 6b medium, whereas the white colonies picked from medium 1 gave either white or red growth on medium 6b. This indicates that the "copper" colonies are masked on medium 1 by the absence of copper in the medium; it tends to prove further that the "copper" bacteria are able to live in the absence of copper in the medium.

The cultures first isolated from colonies were replated, reisolated several times, and retransferred to media 6b and 7. Each reacted true to type. They were separated on the basis of pigmentation characteristics, size and appearance of colony. Thirteen pure cultures of "copper" bacteria were thus selected for further study.

On microscopic examination, various cultures of "copper" bacteria were found to be very small, short rods. Those taken from the pigmented colonies appeared darker and were more readily recognized than the bacteria taken from the white colonies, which had either lost their red-brown pigment or had not acquired it yet. They were found to behave alike toward the gram stains and were gram-negative organisms without any spores.

When plated out, the "copper" bacteria produced colonies both on the surface and below the surface of the agar. The surface colonies pigmented very rapidly, usually within 48 hours, and remained pigmented from two to four days. They were convex, had a very smooth edge and the surface was smooth and shiny. In losing the pigment, the colonies did so in a very characteristic manner. The pigment was gained or lost in the form of concentric zones until finally the colony no longer possessed any pigment at all. This occurred very rapidly in the surface colonies, and often only one concentric ring was noted. In the deep colonies, however, the rate of pigmentation was slower, usually requiring from four to five days to appear; the pigment remained from four to ten days depending on the number of colonies in close proximity to one another. The deeper colonies continued to produce concentric rings of red and white to the extent of seven or eight in number. The areas of white between the rings of red retained a more or less granular appearance. The reason for the occurrence of the rings is not definitely known, but it is thought that the copper nearest the youngest cells, the periphery of the colony, is responsible for the pigmentation, and, as the colony enlarges, this area is no longer adjacent to the fresh supply of copper, and becomes white again.

In the case of streaks of the bacterial cultures, grown on medium No. 7, the pigmentation occurred at the base, the culture continuing to grow along the entire streak. The growth was usually shiny and echinulate in appearance. In time, it became tough and leathery, as did the colonies as well. The culture remained alive but it could be transferred only by removing the whole colony or a mass of growth from the agar. The cultures of the "copper" bacteria died in a relatively short time under aerobic conditions, but not under reduced oxygen pressure. For this reason, the pure cultures are kept under partial anaerobic conditions. This can be accomplished by using deep tubes of soft agar or carragar medium.

CONCLUSIONS

1. The presence of 100 mg $CuSO_4.5H_2O$ in a sea water medium allowed the development of certain red-brown pigmented colonies, from sea water and from bacterial films taken from submerged surfaces. No such colonies were obtained from fresh water or from soil by the use of fresh water media containing the same copper salt.

2. The addition of various forms of copper to sea water had at first a depressing effect upon bacterial development and oxygen consumption. This effect was later overcome, and with time a stimulation resulted. The rapidity of adjustment of the bacteria to the addition of copper depended upon the amounts of copper added to the water. High concentrations often proved toxic to a point where the majority of the bacteria were unable to become adjusted, at least within the short experimental period employed.

3. The consumption of oxygen in sea water by bacteria was parallel to the growth rate of the bacteria, as influenced by the amounts of copper added to the water.

4. Sea water and surface films were found to contain bacteria that have a definite affinity for copper, since they are able to withstand concentrations of 500 mg $CuSO_4.5H_2O$ per liter of medium.

5. When grown upon media containing copper, the "copper" bacteria produce colonies pigmented a red-brown color, whereas no color is noted when the same bacteria are grown in the absence of copper. These "copper" bacteria were also grown in the presence of various forms of copper, including $CuSO_4.5H_2O$, $CuCl_2.H_2O$ and Cu_2O.

6. The pigmentation of the surface "copper" colonies was observed on the plates within 48 hours incubation; it persisted for two to four days. The pigmentation of submerged colonies appeared within four or five days' incubation of the plates and lasted from four to ten days.

7. A number of cultures designated as "copper" bacteria and producing red pigmented growth on media containing copper in the form of $CuSO_4.5H_2O$ were isolated. These bacteria were all small, non-spore forming, gram-negative rods.

8. The red-brown pigment in the sub-surface colonies persisted for a long time; however, in surface colonies it rapidly disappeared, in a characteristic manner, namely through the formation of concentric colorless zones which gradually turned the whole colony colorless.

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CHLOROPHYLL-CONTENT OF MARINE PLANKTON[1]

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INTRODUCTION

With the recent progress of oceanography and the accumulation of data regarding the biological and chemical conditions of ocean water many oceanographers have become interested in correlations which relate to organic production and chemical cycles in the sea (Redfield, 1934; Seiwell, 1935; Harvey, et al, 1935; Fleming, 1940; Riley, 1941b). Since such studies require an accurate knowledge of conditions in the sea it is of prime importance to have some method for measuring the potential synthetic capacity of the sea which can be used in survey work as well as at fixed stations.

One of the best methods for determining organic production in the sea is by measuring the oxygen production of a sample *in situ* (Nielsen, 1937). However, this method is not practicable for general oceanographic work. The time-honored method of counting the number of plant cells is not satisfactory. First, it is a slow and tedious procedure. Second, the results must be expressed in numbers of cells when the size of the cells in different species or organisms is very different. Furthermore, silk bolting cloth which is usually used for collecting the samples on board ship is not a reliable filter. The centrifuge method, also, has fallen into disrepute (Nielsen, 1933). In an attempt to find some way of determining the synthetic potentialities of serial samples Harvey (1934) made acetone extracts of plankton and used the intensity of color as an index of the amount of phytoplankton present by comparison with an artificial standard. Since Harvey used silk bolting cloth for filtration his method contains all the errors of the net methods. Furthermore, the "plant pigments" extracted by Harvey include pigments such as carotenoids which are extractable from many animals as well as plants (Gillam, et al, 1939) so that the "Harvey Unit" is not strictly a phytoplankton unit.

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 200.

Better substances to measure are the chlorophylls. Not only are they strictly plant pigments but they are the enzymes directly responsible for organic production. A measure of the chlorophyll-content of the water is a measure of the photosynthetic capacity of the water. The chlorophyll of plankton has been determined by a few workers. Kreps and Verjbinskaya (1930) determined the chlorophyll-content of net plankton from the Barents Sea. Kozminski (1938) measured the chlorophyll of plankton in Wisconsin Lakes obtained with a Sharpless centrifuge. Riley (1938a) used the Harvey Standard as a chlorophyll standard after calibrating with a pure chlorophyll solution. He used paper for filtration. Gillam, et al (1939) included chlorophyll in their biochemical analysis of net plankton from the North Sea. Krey (1939) collected plankton from the region of Kiel with a "cella" filter and measured its chlorophyll-content spectrophotometrically.

METHOD

A practical method to be used in survey work for determining the chlorophyll-content of plankton has the following requirements:

1. Use of small quantities of water.
2. Rapid filtration.
3. Removal of at least 95 per cent of the phytoplankton.
4. Use of small amount of solvent.
5. Rapid reading of chlorophyll concentration.
6. Use of portable equipment.

The present investigations were carried out on the surface plankton off the pier of the Scripps Institution of Oceanography at La Jolla, California during the summers of 1941 and 1942. Four liters of water were collected in a bucket, poured into a glass bottle, removed to the laboratory, and filtered. After testing many kinds of filters, fine chemical filter paper was selected as the most suitable. With the plankton in this locality this filter retained more than 95 per cent of the microplankton and yet permitted rapid filtration. With the use of a little suction four liters were filtered in 15 minutes. Rapid filtration is possible only if the entire surface of the paper is used. This was effected by supporting the paper on the wire gauze of a Seitz bacteriological filter.

The filter paper with contained plankton was placed in 10 ml of 80 per cent acetone in the dark for one-half hour. The resulting extract was passed through a fine sintered glass filter and examined in a Bausch and Lomb visual spectrophotometer. Absorption of light was measured at wave length of maximum absorption in the red, namely,

at 668 mu with collimator slit width of 0.4 mm. Using the absorption coefficient 82.04 determined by MacKinney (1941) for chlorophyll *a*, the concentration of chlorophyll was computed and expressed as milligrams per cubic meter of seawater.[1] Concurrently with the determinations of chlorophyll, phytoplankton counts were made on samples settled in graduated cylinders. The results are presented in Table I and Figure 43.

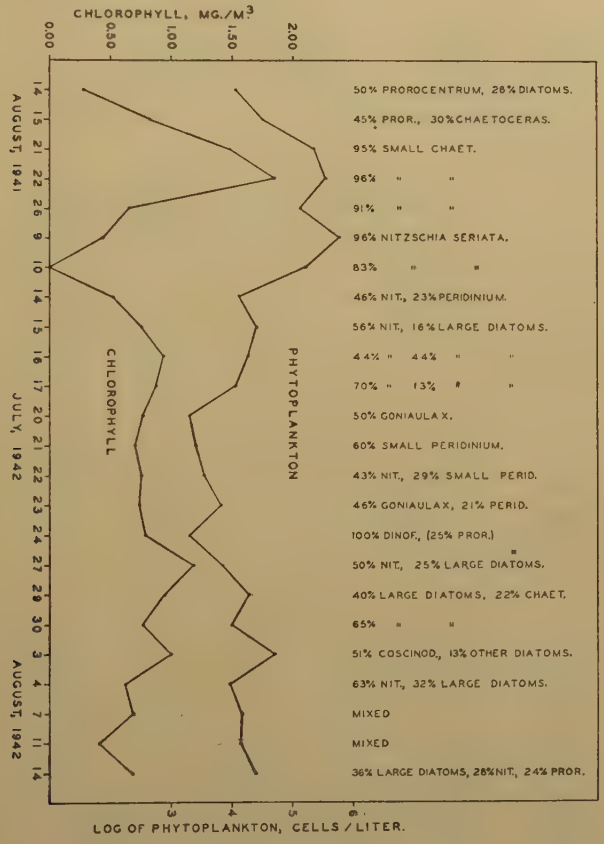

Figure 43.

[1] The absorption maximum of cholorophyll *b* in the red is not far from that of chlorophyll *a* and its concentration in plankton is probably negligible. However, chlorofucine has a maximum somewhat apart, at 627 mu in ether (Strain and Manning, 1942). How abundant this pigment is, is not known but an examination of the raw extract of phytoplankton suggests that it does not occur in large quantity in marine plankton.

This method of determination of plankton chlorophyll satisfies all the requirements listed above except the use of portable equipment. Work is now in progress on the construction of a portable monochrometer especially designed for chlorophyll determinations on board ship.

RESULTS AND DISCUSSION

The concentration of chlorophyll in the plankton at the Scripps pier varied from almost none on July 10, 1942 to 1.86 mg/m³ on August 22, 1941 (Table I and Figure 43). The average value for the twenty-four determinations made throughout the summers of 1941 and 1942 was 0.77 mg. These values are within the range of values found by other workers (Table II).

The phytoplankton counts made at the same time as the chlorophyll determinations varied from 2,000 to 573,000 cells per liter. During the periods of investigation the composition of the plankton was changing continually. The dominant members of the phyto-

TABLE I—Comparison of Total Cell Count, Chlorophyll-content, and Composition of Phytoplankton at La Jolla, California

Date	*Total Cell Count*	*Chlorophyll mg/m³*	*Composition of Phytoplankton*
1941			
Aug. 14	11,600	0.26	50% *Prorocentrum* spp.; 28% *diatoms*
Aug. 15	30,450	0.80	45% *Prorocentrum* spp.; 30% *Chaetoceras* spp.
Aug. 21	219,600	1.46	95% small *Chaetoceras* spp.
Aug. 22	343,160	1.86	96% small *Chaetoceras* spp.
Aug. 26	132,000	0.65	91% small *Chaetoceras* spp.
1942			
July 9	573,000	0.46	96% *Nitzschia seriata*
July 10	168,000	trace	83% *N. seriata*
July 14	13,000	0.53	46% *N. seriata;* 23% *Peridinium* spp.
July 15	25,000	0.75	56% *N. seriata;* 16% large diatoms
July 16	18,000	0.92	44% *N. seriata;* 44% large diatoms
July 17	11,500	0.87	70% *N. seriata;* 13% large diatoms
July 20	2,000	0.76	50% *Goniaulax* spp.
July 21	2,500	0.70	60% small *Peridinium* spp.
July 22	3,500	0.75	43% *N. seriata;* 29% small *Peridinium* spp.
July 23	6,500	0.74	46% *Goniaulax* spp.; 21% *Peridinium* spp.
July 24	2,000	0.78	100% dinoflagellates (25% *Prorocentrum* spp.)
July 27	7,000	1.20	50% *N. seriata;* 25% large diatoms
July 29	18,500	0.93	40% large diatoms; 22% *Chaetoceras* spp.
July 30	10,000	0.76	65% large diatoms
Aug. 3	51,000	1.01	50% *Coscinodiscus* sp.; 13% other diatoms
Aug. 4	9,500	0.62	63% *N. seriata;* 32% large diatoms
Aug. 7	15,000	0.68	Mixed
Aug. 11	14,000	0.40	Mixed
Aug. 14	25,000	0.68	36% large diatoms; 28% *N. seriata;* 24% *Prorocentrum* spp

TABLE II—Comparison of Chlorophyll Determinations in Different Regions by Different Workers. Values in mg/m³

Worker	*Region*	*Depth*	*Filter*	*Mean*	*Maximum*
Kreps and Verjbinskaya, 1930	Barents Sea	upper 25 meters	net	0.02	0.12
Kozminski, 1938	Wisconsin lakes	various	centrifuge	10.0[1]	386.0
Gillam, et al, 1939	North Sea	upper 60 meters	net	0.01	0.135
Krey, 1939	Kiel	upper 30 meters	"cella"	2.00	5.00
Riley, 1938b	Tortugas	surface	paper	0.33	—
Riley, 1939a	Western North Atlantic	surface	paper	1.2	3.6
Riley, 1940	Linsley Pond	upper 13 meters	paper	14.7	40.0
Riley, 1941a	Long Island Sound	surface	paper	17.44	62.0
Riley, 1941b	Georges Bank	surface	paper	5.6	27.2
Graham, 1942	La Jolla	surface	paper	0.77	1.86

[1] Approximate.

plankton community are indicated in Table I and Figure 43 for each day of observation.

The amount of chlorophyll in the plankton should bear a fairly definite relation to the total amount of phytoplankton protoplasm. It can be assumed that the relation between chlorophyll-content and protoplasm may vary widely between species or even according to age or other physiological conditions within a species, so that quantitative determinations of the green pigments cannot be used as an exact measure of plant protoplasm present. However, phytoplankton cell counts are not good indices of the relative degree of biosynthesis in a given sample or region because of the extreme differences in cell size and skeleton weight among the various species. Therefore, chlorophyll assays should afford a more reliable measure of the potential organic synthesis in any given region than the numbers of phytoplankton cells.

The few data available on the chlorophyll-content of phytoplankton species emphasize the differences in species of different sizes. Harvey (1934) estimated that one plant pigment unit was equivalent to 6950–7300 cells of *Rhizosolenia alata* but to only 224 cells of *Biddulphia regia*. According to Krey (1939) one gamma of chlorophyll is equivalent to 286,000 cells of *Chaetoceras gracile*. Our own data indicate that one gamma is equivalent to 150,000–190,000 cells of *C. Van Heurckii*. The minute species of *Nitzschia* contain much less chlorophyll per cell. Pace (1941) estimated that one gamma of chlorophyll was equivalent to 1,840,000 cells of *N. closterium* while our own data show that one gamma is equivalent to 1,250,000 cells of *N. seriata*. It is probable that much smaller numbers of the larger diatoms and dinoflagellates are necessary to supply the equivalent of chlorophyll although data concerning this are not yet available.

Considering the great difference in chlorophyll-content of different kinds of plankton we should not expect a series of phytoplankton counts to vary in accordance with variations in total chlorophyll-content of the plankton. A glance at Figure 43 indicates some general correspondence in the variation of the two factors. A closer examination, however, shows that there is no direct relationship between chlorophyll-content and numbers of cells. If the composition of the phytoplankton is taken into consideration, however, most of the discrepancies can be accounted for.

When the observations were started August 14, 1941 the phytoplankton population was 50 per cent *Prorocentrum*. At that time the chlorophyll-content and the cell count were comparatively low. In a few records the cell counts and chlorophyll-contents rise and fall together until there is an abrupt change in the composition of the plankton from predominately small *Chaetoceras* species to *Nitzschia seriata*, a very small needle-shaped species. While the percentage of *Nitzschia* is high the chlorophyll content is low. As the percentage of *Nitzschia* decreases the amount of chlorophyll increases in proportion to the cell count.

In the next few measurements a medium amount of chlorophyll is maintained by relatively low numbers but, significantly, the phytoplankton community is represented more by dinoflagellates on these days. During the later observations, excepting on July 29, there was a good correspondence between chlorophyll-content and cell count. Mixed phytoplankton communities obtained at those times.

At inshore stations a certain amount of algal detritus probably contributes some chlorophyll. What percentage of the total this may reach has not been determined. It is highly desirable that the present studies be extended to offshore stations when conditions allow.

When portable equipment has been perfected for routine determinations of chlorophyll such work should give values very useful for general oceanographic work. Although it is not intended that chlorophyll determinations should entirely replace cell counts, quantitative chlorophyll data should be more easily interpreted in terms of the chemical conditions of the water than the data regarding the numbers of phytoplankton cells or the amount of acetone soluble pigments of total microplankton. Meanwhile, continued parallel studies of total chlorophyll and of relative numbers of various species of phytoplankton should provide useful information for various branches of oceanographic study.

The writer is indebted to the Director, Dr. H. U. Sverdrup, of the Scripps Institution of Oceanography and to other members of the

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A POSSIBLE FUNCTION OF BIOLUMINESCENCE[1]

By

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It has been found difficult in many cases to conceive a use of the light to the luminescent organism, despite the large number of possibilities of ecological significance which have been suggested (*vide* Mangold, 1910, pp. 326–32; Kemp, 1910, p. 649; Pütter, 1911, p. 487; Harvey, 1920, pp. 81–4; Rauther, 1927, pp. 162–6; Harvey, 1942, pp. 58–87). This difficulty has led to the conclusion by some that bioluminescence does not always have a function. Thus, Harvey (1929, p. 780) says, "who can suggest the use of light to a luminous bacterium, . . . or . . . to a protozoan, living at the surface of the sea, blown hither and thither by the wind. One is forced to the conclusion that in their case the light is merely fortuitous, a chance phenomenon, accompanying some of the chemical changes in the organism." Russell and Yonge (1928, p. 194) follow Harvey (1920) in saying, "it is difficult to see what use luminescence can be to bacteria, Noctiluca, jellyfish or the Sea Pens, it is probably merely a by-product of the normal activities of these creatures." Sverdrup, Johnson and Fleming (1942, p. 834) likewise consider that "In dinoflagellates, bacteria, jellyfish, hydroids, and so forth, there appears to be no possible utility [of the light]."

Against conclusions as to the incidental nature of light produced by animals based upon the above negative grounds, it may be advanced that whereas the luminescence of plants is uncontrolled, the light of protozoa and of practically all higher animals is exhibited only upon stimulation; indeed, Harvey (1932: *vide* Harvey, 1942, pp. 158–9) compares the control of luminescence in protozoa to that of muscle or nerve cell. Further, it appears that light is produced by some protozoa and jellyfish only, or most actively, in the dark (*vide* Harvey, 1942, pp. 173–79). Thus, if luminescence of these protozoa is not to be regarded as a primary process, the assumption is required that the primary process to which the light is incidental occurs only in moments when the animal is stimulated in the dark. Such a process is perhaps

[1] It gives me pleasure to acknowledge helpful criticism of the basic conception from Dr. J. B. Buck of the Department of Zoology, University of Rochester and from Professor G. E. Hutchinson of Osborn Zoological Laboratory, Yale University.

not inconceivable, but it is unknown, and a use for it would be very hard to imagine.

Consequently Harvey, although at first glance appearing to refuse the guidance of "teleology-without-final-cause" (*cf.* Thompson, 1917, pp. 4–5), would seem actually to be strongly impelled by it, in postulating an unknown and doubtful activity because of a difficulty in conceiving the use of a known one. It would therefore appear to be justifiable, by Harvey's own example, to speculate whether a use of luminescence to a planktonic protozoan is in fact inconceivable.

A possibility which does not seem to have been previously considered is, that luminescence may sometimes serve its producer somewhat in the nature of a burgler-alarm. It has been remarked by Mangold (1910, p. 330), "Nun erscheint freilich die Bedeutung des Leuchtens als Lockmittel als ein bedenklicher Vorteil [Brauer], da es in gleicher Weise wie die Beutetiere so auch die Feinde herbeizieht." It might equally well be considered that an exhibition of light by the prey upon attack by a predator might attract an enemy of the predator. Harvey (1920, p. 82) has remarked that the use of light by *Chaetopterus* to frighten a predator would appear ineffectual, since the extracted worm could not rebuild its tube and would in any case be protected only at night. If the luminescence of a *Chaetopterus* attacked by a nocturnal feeder, for example an eel (*cf.* Dahlgren, 1916, p. 689), should assist a secondary predator, for example a dogfish, to locate and capture the eel, then the species although not the individual worm would have received a degree of advantage from its light.

It is conceivable that the luminescent plankton might at night or in the abyss expose predator to predator along the whole length of the food-chain. A peridinian seived by a copepod might by its spark of light facilitate capture of the copepod by a herring (*cf.* Bigelow, 1926, p. 101), or menhaden might be made conspicuous to weakfish by their luminescent wakes. Fishes, as herring or mackerel, feeding upon copepods feeding upon luminescent protozoans might themselves at the same time be exposed to further predators, as bluefish, by the light of the protozoans, to the indirect detriment of the protozoans. However, the efficiency of hunting by sight of a trace such as luminescence of microplankton might be expected to decrease with decrease in density of population, and hence with increase of mean distance between individuals, at ascending levels of the food-chain. There might thus be a net advantage to luminescent plankton from facilitation of the capture of primary predators, despite the facilitation also of the capture of secondary, "protective" predators by further members of the food-chain. From the complexity of the food-chain alone, such hypothetical

net gain from luminescence would be exceedingly difficult to estimate, but it might nonetheless be real enough.

It is not even known at present whether the premise fundamental to the above hypothesis, namely, that there are predators capable of hunting their prey by the light which the latter excite in other organisms, is valid. However, some consequences deducible upon the assumption that luminescence as a "burglar-alarm" plays a significant part in maintaining the balance between prey and predator in the sea may be mentioned, as capable of investigation or bearing upon unresolved problems.

For maximum efficiency, luminescence serving as an alarm might be expected to differ in color, form, intensity, duration, or mode of evocation at different levels of the food-chain and according to the different forms of predation and habits of the prey. Thus, light of the lowest intensity, briefest duration, and readiest display might be on the whole most useful to the smallest prey, as making distinguishable to least range the wake of "protective" predators. Species fed upon chiefly by predators capable of visual guidance might on the whole benefit more by their luminescence if solitary than gregarious, since the light might signal to the primary as well as to secondary predators.

Secondary predators hunting by the luminescent alarm might cruise most advantageously in such spatial relation to the normal level of the signalling organism as would best permit detection of the light. Thus, in contrast to scent or touch of the primary predator, the light of the prey might best be perceived or distinguished by a secondary predator from a different instead of from the same horizontal level as is frequented by the hunted forms.

Non-luminescent planktonic forms occurring with light-producing ones might in some circumstances benefit with the latter in protection afforded by the light, a possibility perhaps bearing upon the erratic distribution of the power of luminescence.

Habits of bathymetric range or of vertical migration permitting neutralization of the diurnal and lunar cycles might enhance the value of the luminescent alarm both to its bearer and to associates; as might special habits of feeding or movement by predators in some circumstances negate it.

The production of light resembling a given style of luminescent alarm might be an effective type of luminescent lure, attracting "protective" predators into range without clearly identifying the form employing the lure to its own predators.

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GRAPHIC REPRESENTATION OF SALINITY IN A TIDAL ESTUARY

By

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In any study of organisms in tidal estuarine areas, such as the oyster and its associates, the salinity of the water is of recognized importance as a factor limiting the distribution and controlling the activity (movement, feeding, spawning) of the various species.

All previous graphic methods of presenting salinity data, such as the construction of isochlors or the spotting of a single series of samples, are deficient in that they in no way indicate the variations and extremes which can occur. Since biological activity is greatly altered by such extremes a more inclusive technic of presentation is desirable.

In Figure 44 this has been done for the oyster-growing areas of the New Jersey waters of Delaware Bay. This haligraph combines a map and graph on which are plotted not only the points from which water samples were obtained and the recorded salinity of these samples, but also the position of such samples with reference to the total range of salinity as it varies with the tide or the depth. The length of the line which denotes the extent of variation with the tide has been determined for many of the stations by actually obtaining hourly samples over a complete tidal cycle (12–13 hours).

From the extremes so obtained we can construct lines on the map which show the position of penetration of any given salinity. These have been called hyperhals and hypohals. Hyperhal 10, for example, is defined as that point where the salinity just reaches 10 parts per mille during the saltiest part of the tidal cycle (usually between high water and the first hour of ebb in these areas of Delaware Bay). Hypohal 10 represents that point where the salinity falls just to 10 p. p. m. during the freshest part of the tidal cycle.

The completed haligraph can then be compared with a weather map except that changes will not occur with such rapidity. Knowing the temperature of the water and the duration of any given set of haligraphic conditions we are able to compare field with laboratory

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[2] The author wishes to express his appreciation for the many valuable suggestions of Dr. Thurlow C. Nelson, whose interest made this work possible.

Figure 44. Haligraph of Delaware Bay on April 20, 1939. The circles in the upper or map portion represent the positions of the stations sampled. Heavy solid or dotted lines connect points of approximately equal salinity. In the graph portion a circle designates the salinity of the respective sample. The length of the heavy vertical line drawn tangential to each of these circles denotes the range of salinity occurring at this station. The arrow and the position of the circle along the vertical line indicates the approximate stage of the tide (arrow pointing up—ebb tide; down—flood tide). When the heavy vertical line is to the left, the circle indicates the salinity of water taken at the surface (see station 11A); when to the right, of water taken 1 foot from the bottom.

data on such problems as the duration and the degree of salinity necessary to maintain life.

In Figure 44 conditions were probably unfavorable to oyster life above hyperhal 10. Yet, because of the low temperatures prevailing during these adverse conditions no appreciable mortality occurred except at station 31. When similar conditions occurred in July and August of 1938, with water temperatures fluctuating around 25° C., oysters died from station 24 to station 31 in numbers which roughly varied inversely with the salinity of the water. During this same period of low salinities and high temperatures oyster drills (*Urosalpinx cinerea*) were destroyed at points above hypohal 10 except for a few small foci at stations 21, 22 and 26. At the same time oyster drill activity was inhibited at all stations where large scale trapping of this pest was in progress. All these stations had hypohals lower than 15. Traps were also out at the time indicated for Figure 44 (April 20, 1939) and although water temperatures were much lower (9° C.) it is still possible to demonstrate the greater inhibition of drills under conditions of hyperhals less than 15 than with hyperhals greater than 15.

The haligraph for September 22, 1932 clearly shows the effect of drought conditions on the salinity of Delaware Bay.[3] The position of hypohal 15 on this occasion was over 26 miles farther up the bay than on April 20, 1939 or above station 31 on Figure 44. Since extremes almost as great as these can occur within a few months (e. g. between April and August, 1939) we get a very clear picture of the variations in environment to which organisms living in these waters are exposed.

The recording of mean salinities can have but little meaning in biology. Brackish water species are kept within certain spatial limits by the effects of the extremes of salinity and of their duration, not by the means.

It is hoped that the haligraph will facilitate work on similar problems and that refinements of expression can be added in the future.

[3] Many thanks are due Mr. J. Richards Nelson for the use of these data which were gathered when he was in charge of this station.

TO AUTHORS

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